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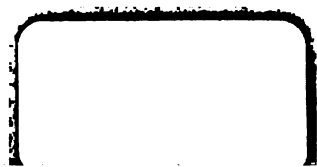
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THIRTY-SIXTH ANNUAL REPORT

OF



THE LOCAL GOVERNMENT BOARD,

1906-07.

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SUPPLEMENT

CONTAINING THE

REPORT OF THE MEDICAL OFFICER

For 1906-07.

NEW YORK  
PUBLIC

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Presented to both Houses of Parliament by Command of His Majesty.

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1908

**PUBLIC HEALTH.**

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**ANNUAL REPORT**

**OF THE**

**MEDICAL OFFICER**

**OF**

**THE LOCAL GOVERNMENT BOARD**

**FOR THE YEAR**

**1906-07.**



# CONTENTS.

	PAGE
<b>MEDICAL OFFICER'S REPORT:</b>	
1.—SUPRA-DEPARTMENTAL BUSINESS     ...     ...	viii
2.—ADMINISTRATION IN RELATION TO FOODS     ...	viii
3.—GENERAL ADMINISTRATIVE BUSINESS OF THE MEDICAL DEPARTMENT     ...     ...     ...	ix
4.—VACCINATION AND PUBLIC VACCINATION     ...	x
5.—PLAGUE     ...     ...     ...     ...     ...     ...	xiv
6.—CHOLERA     ...     ...     ...     ...     ...     ...	xv
7.—AUXILIARY SCIENTIFIC INVESTIGATIONS     ...	xv

## APPENDIX A :

No. 1.—Summary of the Work of the Medical Inspectors during the year 1906     ...     ...     ...     ...	1
No. 2.—Abstract of Medical Inspections made in 1906 with regard to the Incidence of Disease on particular places, and to questions concerning Local Sanitary Administration     ...     ...	7
No. 3.—Local Housing Conditions and Administration, illustrated by Extracts from Reports made by Medical Inspectors in 1906-7     ...     ...	31
No. 4.—Report by Dr. Thorne on the Operations of the Animal Vaccine Establishment at Lamb's Conduit Street during the year 1906-7     ...	65
No. 5.—Report by Dr. Blaxall on the Operations of the Glycerinated Calf Lymph Establishment, 1906-7     ...     ...     ...     ...     ...	66
No. 6.—Digest of the Vaccination Officers' Returns with regard to Children whose Births were registered in the Year 1905     ...     ...     ...	68
No. 7.—Summary by Dr. Bruce Low of the Progress and Diffusion of Plague throughout the World during 1906     ...     ...     ...     ...	76
No. 8.—Report by Dr. Bruce Low on the Manifestations of Cholera throughout the World during 1906	111

	PAGE
<b>APPENDIX B :</b>	
No. 1.—Report by Dr. Klein on Further Research in connexion with Plague ... ..	123
No. 2.—Observations by Drs. Andrewes and Gordon on some of the Defensive Mechanisms of the Body against Pyogenic Cocci ... ..	141
No. 3.—Report by Dr. Andrewes on the Micro-organisms present in Sewer Air and in the Air of Drains ... ..	183
No. 4.—Report by Dr. Savage on Bacterial Studies of Milk from Healthy and Diseased Cows ...	205
No. 5.—Report by Dr. Savage on the Distribution of Organisms of the Gaertner Group in the Animal Intestine ... ..	253
No. 6.—Report by Dr. Horder on a Study of the Micro-organisms associated with Rheumatic Fever and Malignant Endocarditis ... ..	279
No. 7.—Preliminary Report by Dr. Sidney Martin on the Action of the <i>Streptococcus Fæcalis</i> and of its Chemical Products ... ..	311

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**APPENDIX C :**

Preliminary Report by Dr. Blaxall and Mr. Fremlin on the results of sustained subjection of Glycerinated Calf Lymph to Temperatures below Freezing Point ... ..	317
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# REPORT

TO THE RIGHT HONOURABLE JOHN BURNS, M.P.,  
PRESIDENT OF THE LOCAL GOVERNMENT BOARD.

SIR,

I HAVE the honour to submit a record of the work carried out in the Board's Medical Department in 1906-7.

MEDICAL  
OFFICER'S  
REPORT.

This record is concerned entirely, as will also be the greater part of the next Annual Report, with the work of the Medical Department during the tenure of office of Mr. now Sir W. H. Power, K.C.B., F.R.S., and I submit it, therefore, without any lengthy comment. Sir William Power retired in January, 1908, his period of office having been brilliant in scientific advancement and marked by great sanitary amelioration throughout the country. Throughout his association with the Board, first as Medical Inspector for 17 years and afterwards as Assistant and Chief Medical Officer for nearly 16 years, Sir William Power's influence both in initiating and in inspiring work of far-reaching scientific and practical importance impressed itself in every branch of the Board's medical administration; and his contributions to the annual reports of the Board's Medical Officer have become classic in the literature of preventive medicine and landmarks in its history. Public Health retains the advantage of Sir William Power's continued services as Chairman of the Royal Commission on Tuberculosis, to which post he was appointed in succession to Sir Michael Foster, K.C.B., in 1907. Sir William Power is continuing also his work as a Member of the General Medical Council, and as a Member of the Royal Commission on Sewage Disposal.

During the year 1906-7 the following events affecting the personnel of the department occurred. Dr. Buchanan continued to act temporarily as inspector of foods and to develop administratively, under the medical officer, the sub-department which had been created during the previous year. The work of this sub-department is mentioned again on page viii. The vacancy thus created on the staff of medical inspectors continued to be filled by Dr. J. Spencer Low, who had acted in the same capacity during the previous year. Mr. G. C. Hancock, assistant medical officer to the port of London, during the earlier months of the year, was a medical inspector during Dr. Theodore Thomson's service under the Foreign Office in a sanitary mission to the Persian Gulf. Mr. Thomas Stott, Director of the animal vaccine establishment, died during the year and you appointed as his successor Dr. L. C. Thorne Thorne. Mr. A. B. Farn, assistant inspector attached to the national vaccine establishment,

**MEDICAL  
OFFICER'S  
REPORT.**

attained the prescribed limit of age and vacated his appointment. Mr. W. F. Mulcahy, the laboratory clerk attached to the glycerinated lymph laboratories, died during the year. The vacancies arising through the appointment of Dr. Thorne, the retirement of Mr. Farn, and the death of Mr. Mulcahy were not filled up in view of the concentration of work at the new laboratories, which were in course of construction at the end of the year to which this report relates.

**SUPRA-DEPARTMENTAL BUSINESS.**

During the year under report Sir William Power gave evidence before the Royal Commission on Vivisection. As heretofore he advised on public health questions affecting this country's Dependencies and Colonies, which have been referred to the Board by the Foreign Office and by the Colonial Office, and similarly he was consulted respecting legislative measures contemplated by the Privy Council and bearing directly or indirectly on health questions.

Dr. Parsons again acted in 1906-7 as the Board's representative upon the Committee of Advice on the Geological Survey.

He gave evidence on May 10th, 1906, before the Select Committee of the House of Commons on the Small Holdings Bill on the subject of Building Byelaws in Rural Districts. Dr. Parsons, in conjunction with Mr. Kitchin, the Board's Architect, also commenced an enquiry into the construction of isolation hospitals, with the view especially of ascertaining how the essential requirements of such hospitals could be met without excessive cost.

Dr. Bruce Low has continued to serve on the Committee which was appointed by the Advisory Board for the Army Medical Services "to direct a course of investigation into the practical prophylactic and therapeutic value of current methods of immunisation against enteric fever." This investigation is still proceeding.

**ADMINISTRATION IN RELATION TO FOODS.**

Last year's report referred to the considerations which led to the formation of a special sub-division of the Medical Department for duty in relation to questions affecting the purity and wholesomeness of the food supplies of the country.

In pursuance of this policy during the year under review, Dr. Buchanan continued to act as inspector of foods, Dr. A. W. J. MacFadden being appointed in April, 1906, as assistant inspector of foods. The work done by these two officers included inquiries in regard to certain local and general administrative questions arising under the Sale of Food and Drugs Acts and various communications with public analysts and other local officers concerned with the collection and examination of foods under those Acts. Special reference may be made to a report

by Dr. MacFadden regarding the contamination of tartaric acid, citric acid, and cream of tartar by lead and arsenic, and to the issue by the Board in July, 1906, of a circular to authorities under the Sale of Food and Drugs Acts advising action to be taken to prevent the addition of preservatives to milk. During the year 1906-7, much of the inspectors' time was occupied with questions relating to the wholesomeness of imported foods (especially meat foods and canned foods) and with a variety of other subjects, which received consideration by the Board, in connection with the Bill relating to food regulations which you introduced into the House of Commons in 1906. Dr. Buchanan's work on these subjects was continued in 1907, when the Public Health (Regulations as to Food) Act was passed, and most of it was incomplete at the end of the year under review. The work done in the sub-department during the years 1906-7 and 1907-8 will accordingly be dealt with in a single report by Dr. Buchanan, which will form part of the next annual report of the medical officer.

MEDICAL  
OFFICER'S  
REPORT.

#### GENERAL ADMINISTRATIVE BUSINESS OF THE MEDICAL DEPARTMENT.

As in past years your Medical Department sought to extend its usefulness by obtaining and disseminating knowledge. Information and experience in matters relating to public health have been exchanged with other Departments of Government, and with representatives of a number of authorities and public bodies at home and abroad. Conferences have been held at Office with local authorities and their officers with reference to prevalences of disease, hospital construction, local byelaws, appointment of officials and the like. Advice and assistance have been directly rendered to local authorities by visits of medical inspectors. Formal inquiries have been held by these officers into such matters as combination of districts for hospital or other administrative purpose, loans for hospital sites and buildings, proposals as to local byelaws, and schemes for water supply and sewage disposal.

A summary of the work of the medical inspectors is given in Appendix A., No. 1, and in Appendix A., No. 2, will be found abstracts of the several detailed inquiries undertaken by the inspectors on account of outbreaks of infectious disease or of defective sanitary administration, and references to published reports which have been issued on these matters.

The reports enumerated in the Abstract on pages 7 to 30 have already been printed and circulated, as indicated in the Abstract, and have formed the subject of local comment in the districts specially concerned. In some instances they have been the means of securing much needed improvements in sanitary administration.

Portions of these reports dealing with matters of public concern are printed on pages 31 to 64. Dr. Darra Mair's report

HOUSING.

**MEDICAL  
OFFICER'S  
REPORT.**

is partially reproduced without the illustrations in the original report (No. 262), which is on separate sale. It draws attention to conditions of housing in Whickham Urban District, and in other parts of Durham and Northumberland which deserve wide publicity. The problem of housing is also dealt with in reports by Drs. Copeman, Fletcher, Mivart, Farrar, Spencer Low and others of the medical inspectors, and extracts from some of these reports are given on pages 49 to 59.

**HOUSING.**

The closely allied question of the lodging and accommodation of hop-pickers, and of pickers of fruit and vegetables, was dealt with by Dr. R. Farrar in a report (No. 252), which is being followed by further investigations, still in hand, on the conditions of housing of the nomadic classes generally, including navvies on large works of construction.

**TENURE OF  
OFFICE.**

The bearing of reasonable security of tenure of office of sanitary officials on efficiency of work is most intimate, and in this connection the extract from Dr. R. J. Reece's report upon the sanitary administration of the Trowbridge Urban District is instructive (*see* page 59).

**VACCINATION AND PUBLIC VACCINATION.**

*Inspection of Vaccination.*

During 1906, 271 unions, comprising 1,440 vaccination districts, were inspected in regard to vaccination. The Public Vaccinators of 963 of these districts were recommended for award under section 5 of the Vaccination Act, 1867, the total sum awarded being £20,816 7s.

*Animal Vaccine Establishment.*

The director of the Animal Vaccine Establishment reports (Appendix A., No. 4) that during the year ending 31st March, 1907, the number of vaccinations performed at the Board's Station, Lamb's Conduit Street, was 986. All were primary vaccinations.

*Glycerinated Calf Lymph.*

Dr. Blaxall reports (Appendix A., No. 5), that during the twelve months ending 31st March, 1907, 551,750 charges of glycerinated calf lymph were issued from the Board's Laboratory. The lymph issued by the Board in 1906-7 maintained its customary high quality; in primary vaccination the "case success" was 99 per cent., and the "insertion success" 94·7 per cent.

In Appendix C. will be found a preliminary report by Dr. Blaxall and Mr. Fremlin on the results of sustained subjection of glycerinated calf lymph to temperatures below freezing point. The investigation has an important bearing on the ability of the Government Lymph Establishment to supply lymph under the pressure of a small-pox epidemic; and investigations on the same lines are being continued.

### *Vaccination Officers' Returns.*

MEDICAL  
OFFICER'S  
REPORT.

Appendix A., No. 6, contains tables compiled from the annual returns of vaccination officers, with respect to infants whose births were registered in 1905. In view of the recommendations of Select Committees of the House of Commons on Parliamentary Publications, the tables in the appendix no longer comprise the vaccination returns for individual provincial Unions. It is proposed to substitute a quinquennial return for the annual return now discontinued.

The following tables are a continuation of those published in previous annual reports :—

#### *England and Wales.*

Observance of the Vaccination Laws in England and Wales as a whole in 1898 and in subsequent years.

Year.	Births.	Vaccinated.	In susceptible.	Had Small-pox.	Exempted.	Died Unvaccinated.	Postponed.	Remaining.	Not accounted for (including cases postponed). Per cent. of Births.
1898 ..	923,059	562,737	3,332	4	47,423	110,912	16,921	181,830	21·5
1899 ..	929,189	617,113	5,579	4	33,573	113,516	16,606	142,999	17·2
1900 ..	927,222	636,940	2,261	2	39,699	103,538	14,225	130,557	15·6
1901 ..	929,882	664,366	2,631	27	39,925	103,007	12,317	108,909	13·0
1902 ..	940,509	703,721	3,027	27	33,759	90,826	12,213	96,336	11·6
1903 ..	948,383	714,637	2,573	17	37,675	91,754	12,489	89,238	10·7
1904 ..	945,500	711,501	2,676	22	40,461	94,696	12,733	83,428	10·2
1905 ..	929,540	705,040	2,252	8	44,309	84,712	13,175	79,984	10·0

#### *England and Wales.*

“Abstention” (legal and illegal) from Vaccination and “Acceptance” of Vaccination in England and Wales in 1893-97 and in subsequent years.

	Per cent. of Births in each instance.								
	1893-97.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.
ABSTENTION. ("Exempted," "postponed," and "remaining"). (Exemptions alone)...	21·0	26·6	20·8	19·9	17·3	15·2	14·7	14·5	14·8
ACCEPTANCE. (Vaccinated) ...	67·7	61·0	66·4	68·7	70·8	74·8	75·4	75·3	75·8

*55 Counties of England and Wales.*

Status (numerically) of COUNTIES in regard of percentage of Births Vaccinated in 1893-97 and in the year 1905.

Grade.	Percentage of Births Vaccinated.	Counties in each Grade.			
		1893-97 (antecedent to Act of 1898).		1905.	
		Number.	Per cent. of total Counties.	Number.	Per cent. of total Counties.
I. {	90 and upwards ...	Nil	} 34.5	Nil	} 50.9
	85-90 ...	6		16	
	80-85 ...	13		12	
II. {	75-80 ...	8	} 30.9	10	} 34.5
	70-75 ...	9		9	
III. {	65-70 ...	6	} 18.2	2	} 7.3
	60-65 ...	4		(c) 2	
IV. {	50-60 ...	(a) 6	} 16.4	(d) 1	} 7.3
	Less than 50 ...	(b) 3		(e) 3	

(a) Derby.  
Gloucester.  
Norfolk.  
Notts.  
Wilts.  
Radnor.

(b) Bedford.  
Leicester.  
Northants.

(c) Gloucester.  
Wilts.

(d) Derby.

(e) Bedford.  
Leicester.  
Northants.

*Metropolitan Unions.*

"Abstention" (legal and illegal) from Vaccination, and "Acceptance" of Vaccination, in the METROPOLIS as a whole in 1893-97 and in subsequent years.

	Per cent. of Births in each instance.								
	1893-97.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.
ABSTENTION. ("Exempted," "post- poned," and "re- maining.")	23.9	34.4	28.7	26.8	25.2	22.1	21.7	20.2	20.1
(Exemptions alone)...	—	1.4	1.0	1.0	1.1	0.8	1.0	1.1	1.2
ACCEPTANCE (Vaccinated) ...	65.4	53.8	58.8	61.7	64.3	68.0	68.9	69.7	70.7

## 31 Metropolitan Unions.

MEDICAL  
OFFICER'S  
REPORT.

Status (numerically) of Unions in the METROPOLIS in regard of percentage of Births Vaccinated in 1893-97 and in the year 1905.

Grade.	Percentage of Births Vaccinated.	Unions in each Grade.					
		1893-97.			1905.		
		No.	Names.	Per cent. of Total Unions.	No.	Names.	Per cent. of Total Unions.
I.	90 and upwards.	Nil	—	—	Nil	—	—
	85-90	2	Whitechapel .. Woolwich ..	19'4	4	St. George's, Han- over Square. Kensington .. Westminster .. Woolwich ..	32'5
	80-85	4	St. George's Han- over Square. .. Greenwich .. Hampstead .. Paddington ..		6	Chelsea .. .. Greenwich .. Hammer-smith .. Hampstead .. Marylebone, St. [Whitechapel] ..	
	75-80	5	Chelsea .. .. Fulham .. .. Hammer-smith .. Kensington .. London, City of ..		6	Fulham .. .. Lewisham .. London, City of .. [Paddington] .. Strand .. .. Wandsworth ..	35'5
II.	70-75	6	Lewisham .. .. Marylebone, St. .. St. Pancras .. Strand .. .. Wandsworth .. Westminster ..	35'5	5	Bermondsey .. St. George-in-the- East. .. .. St. Giles .. .. Islington .. .. Lambeth .. ..	
	65-70	6	St. George-in- the-East. .. St. Giles .. .. Holborn .. .. Islington .. .. Bermondsey .. Southwark ..		3	Camberwell .. Holborn .. .. [St. Pancras] ..	16'1
	60-65	2	Camberwell .. Lambeth .. ..		2	Hackney .. .. [Southwark] ..	
	50-60	2	Poplar .. .. Stepney .. ..	19'4	2	Shoreditch .. Stepney .. ..	16'1
IV.	less than 50	4	Bethnal Green .. Hackney .. .. Mile End .. .. Shoreditch ..		3	Bethnal Green .. Mile End .. .. [Poplar] .. ..	

Italics signify ascent, [ ] descent, in grade.

The returns show a slight increase in the percentage of births vaccinated, and of infants exempted under certificates of conscientious objection. At the time of making the return, which as in past years was in February, only 10 per cent. of the births registered in 1905 were "not finally accounted for" as regards compliance with the law.

## PLAGUE.

In 1906 plague continued to prevail in all quarters of the globe. In India there was, however, diminution in the ravages of the disease. Dr. Bruce Low in his annual summary of the progress and diffusion of plague throughout the world (Appendix A., No. 7), states that the reported deaths from plague in India in 1906 numbered only 356,721, as compared with 1,069,140 in 1905 and 1,112,376 in 1904. The causes which led to this marked reduction are not yet apparent, and unhappily the decline in the death-rate from plague has not been maintained, the year 1907 bidding fair to exceed any previous record of fatal plague in India. The provinces which suffered most during 1906 included the Punjab, Bombay, Bengal, and the United Provinces of Agra and Oudh.

In other parts of Asia plague continued to manifest itself. A sharp outbreak occurred in the Seistan province of Persia; and in China there were outbreaks of the disease at Hong Kong, Canton, Swatow, Foochow, and Amoy. In Japan sporadic cases appeared in several localities, but in Formosa, where plague has prevailed for over ten years, it proved more severe in 1906 than in 1905. Minor outbreaks were reported from Siam, French Indo-China, the Straits Settlements, the Federated Malay States and the Philippine Islands.

There was no actual epidemic in Australia, but scattered cases occurred in New South Wales, Queensland, and Western Australia; also in New Caledonia and the Hawaiian Islands.

South America continued to be infected by plague, outbreaks occurring in various towns and districts in Brazil, Argentina, Paraguay, Chili and Peru.

In South Africa the disease has almost ceased to manifest itself. A few cases still continue to appear in Central Africa in the endemic region near Lake Victoria Nyanza. In Mauritius where plague has been annually epidemic during the last eight or nine years the 1906 outburst was of average severity, and was largely confined to Port Louis, the capital of the Colony.

Egypt showed in 1906 a slight increase in the number of notified cases of plague. In Turkey limited outbreaks of the disease occurred at Jeddah, Trebizond, Adalia and Beirut. This disease also appeared at Koweit in the Persian Gulf. As regards Europe, there was an outbreak of plague in Astrachan in European Russia; and a few cases of ship-borne suspected plague were reported at several ports in the Mediterranean and elsewhere, though in only one instance, at Trieste, was bacteriological confirmation of the diagnosis forthcoming.

In England and Wales no actual plague occurred, though several ships arrived at our ports technically infected, having

had a case or cases of plague on board, either while in the port of departure or shortly after sailing from the foreign port. Details of the outbreaks of plague throughout the world, so far as information was obtainable respecting them, will be found in Dr. Bruce Low's report appended.

MEDICAL  
OFFICER'S  
REPORT.

### CHOLERA.

Unlike plague, cholera showed in India a considerable increase instead of diminution during 1906, the total deaths from this cause numbering 713,664, as compared with 441,786 in 1905, and 193,657 in 1904. The provinces which suffered most during the year were Bengal, the United Provinces of Agra and Oudh, Madras and Eastern Bengal & Assam. The details of these occurrences are given in Dr. Bruce Low's appended report on the Manifestations of Cholera throughout the world during 1906 (Appendix A., No. 8). Outside India, so far as can be learnt, there were no large epidemics of cholera during the year; but the disease appeared in Ceylon, Siam, the French East Indian Possessions, the Straits Settlements, the Federated Malay States, and in the Philippine Islands. There was a sharp outbreak at Shanghai, and localised groups of cases were reported from Hong Kong, Foochow, and Hankow. There was no cholera in Europe in 1906, except in Russian Poland, where in the early months of the year a continuation of the 1905 epidemic in the basin of the Vistula was observed. The disease had completely disappeared from Poland by March.

No cholera cases were reported in England and Wales in 1906, but three ships technically infected by the disease arrived at English ports each having had a case of cholera on board while in an eastern port before commencing the homeward voyage.

### AUXILIARY SCIENTIFIC INVESTIGATIONS.

In 1905-1906 Dr. Klein showed that the dried necrotic organs of guineapigs dead of subacute plague possessed decided prophylactic potency for rats, in-as-much as the injection of watery extract of those organs immunises rats against a subsequent fatal infection. In order to preserve and transmit this prophylactic in a reliable and sterile form, a number of experiments were made during 1906-1907 which showed that the clear filtrate of the dried organ-prophylactic can be sterilized by heat and preserved in sealed phials for several months without losing its prophylactic potency.

PLAGUE  
PROPHY-  
LACTIC.

In the second portion of his report Dr. Klein deals with the preparation of a therapeutic agent. In a former report he had shown that in plague—unlike some other infectious diseases (cholera, diphtheria)—the blood of an animal which had passed through and recovered from an attack of plague does not contain in appreciable amount antibodies able to neutralise the

MEDICAL  
OFFICER'S  
REPORT.

action of the specific microbe. It was, however, thought probable that the organs usually most affected might contain the desired antibodies in an animal which had recovered from plague or which by previous prophylactic preparation had been rendered immune against plague. To put this proposition to the test experiments were made with the organs of a monkey, guineapigs, rabbits, and rats. As a result it was found that the watery extract of the liver and spleen of a rabbit which had recovered from a somewhat severe attack of plague had therapeutic potency in a satisfactory degree. 100 milligrams of a watery emulsion (in 1 dose or 50 milligrams in 2 doses) injected 24 hours after infection with *B. pestis* had a pronounced therapeutic effect on rats.

DEFENSIVE  
MECHAN-  
ISMS  
AGAINST  
PYOGENIC  
INFECTIONS.

Drs. Andrewes and Gordon contribute a report on the defensive mechanisms of the body against infection by the pyogenic cocci, which forms a continuation of the reports on these cocci appearing in previous volumes. The most extensive section of their communication refers to the question of bacteriolytic defence. While admitting that the chief means of defence against these cocci is a phagocytic one, Drs. Andrewes and Gordon bring forward evidence that the serum of normal, and still more, of immune animals is not so devoid of bacteriolytic power as has been hitherto supposed. Their method of testing this, though not new in principle, is new in some of its details, and appears to demonstrate a not inconsiderable degree of bacteriolysis during the early hours of contact between the blood or serum, and *staphylococcus pyogenes aureus*. They describe also a certain number of observations upon the course of experimental infection of rabbits with the same organism, which suggest that successful defence may depend in part upon the readiness with which the body can bring its leucocytes into play.

THE BAC-  
TERIAL  
CONTENTS  
OF DRAIN  
EFFLUVIA.

Dr. Andrewes' report on the micro-organisms present in sewer air and in the air of drains is on a subject of great practical importance, and the continuation of his experiments, which will be published shortly, still further emphasises their practical bearings. So far as the investigation now reported upon is concerned, Dr. Andrewes has shown that under many ordinary circumstances sewage bacteria are to be found in the air of drains and sewers, the view that sewage does not readily give up its bacteria to sewer air being shown to be incorrect.

BACTERIAL  
CONTAMINA-  
TION OF  
MILK.

Dr. Savage submits a report dealing with the bacterial contamination of milk, which is mainly concerned with the bacterial and cellular content of milk as obtained from healthy cows and under conditions which, in the main, exclude all bacteria other than those which may be present in the udder.

The examinations showed that staphylococci and streptococci were present in the vast majority of the samples, the former being especially abundant. The streptococci present were of

many kinds and as a class showed considerable differences from the streptococci of cow dung. One particular kind of streptococcus, a long chain form, possessing more or less definite biological properties, was found to be present in a number of the samples and in all three cases of ulcerated teats. In Dr. Savage's opinion there was probably an etiological connection between the presence of this streptococcus and the local teat condition.

**MEDICAL  
OFFICER'S  
REPORT.**

*Bacillus coli* and glucose fermenting organisms in general, were almost invariably absent from milk samples obtained direct from the udder. This fact is important as indicating that when *B. coli* is found in milk samples it probably gained access to the milk *after* it left the teats.

The number of leucocytes in the milk samples varied from 14 to 4,100 per cubic millimetre. It was found that the number of leucocytes was increased in advanced stages of pregnancy and in local inflammatory conditions of the milk producing organs and ducts, and that increase in their number might also be due to some antecedent injury or inflammation of the udder all other traces of which might have completely disappeared.

A commencement was made with the examination of milk samples obtained from cows suffering from definite inflammatory mischief, a number of cases of garget or mastitis in cows being examined. The bacteriological and particularly the cellular content of the milk in these cases was markedly different from the milk of normal cows. In several of these a long chain streptococcus was present in great abundance very similar in its characters to the streptococcus isolated from the cows with ulcerated teats and from some of the apparently healthy cows. This streptococcus in its biological attributes closely approximated to one of the types of streptococcus found to have a special connection with inflammation of the fauces and with scarlet fever in man. This relationship suggests a possible association between these conditions in the cow and those in man which will receive further investigation.

Dr. Savage also contributes a report dealing with the distribution of the organisms belonging to the Gaertner group in the animal intestine. These, as is well known, include the bacilli causing most cases of meat poisoning, and the bacilli of paratyphoid fever, and those associated with a number of diseases in the lower animals.

**THE DISTRIBUTION OF  
GAERTNER  
BACILLI.**

The object of the investigation was to throw light upon the actual source of such bacteria, and in particular to ascertain to what extent they were natural inhabitants of the animal intestine.

The first part of the investigation was concerned with an inquiry into the most suitable methods for the isolation of this group of organisms from the many allied bacteria present as natural intestinal inhabitants.

**MEDICAL  
OFFICER'S  
REPORT.**

Direct plating of the material to be examined upon certain special selective coloured media was found to be of considerable value, but on the whole "enrichment methods", in which dulcitate malachite green broth of a definite composition was employed, gave the best results. A combination of direct plating and the use of this enrichment medium was eventually employed.

The remainder of the report deals with the results of the examination of the intestinal contents of certain healthy animals. The intestinal contents, and more particularly scrapings from the mucous membranes of both small and large intestine of a series of animals, were examined. A very large number of organisms were isolated and investigated. In three bullocks examined no organisms resembling the bacilli of the Gaertner group were isolated.

From six pigs a number of bacteria were isolated somewhat closely resembling the bacilli of this group; but these on further investigation and more particularly by the employment of certain additional tests, could be culturally distinguished. In addition, none of them reacted with appropriate specific sera.

The only calf examined showed abundant bacilli, which, with the tests in vogue in most laboratories, were indistinguishable from true Gaertner bacilli, and which were possessed of considerable pathogenicity. They were, however, to be distinguished from the known pathogenic members of this group by their agglutination properties, and by their failure to ferment salicin. The significance of these results can only be considered after a more extended investigation and the examination of a larger series of animals.

**THE MICRO-  
ORGANISMS  
ASSOCIATED  
WITH  
RHEUMATIC  
FEVER.**

Dr. Horder has contributed a careful study of the micro-organisms associated with rheumatic fever. This disease is one of the chief causes of disablement in active life, and the importance of its study is very great. Dr. Horder's chief results are negative. He shows that, using the same methods as have been employed by others who have secured positive results, no micro-organism could be found in the blood of rheumatic fever patients; and he inclines to the view that in the cases where other observers have found such micro-organisms in the blood there was the superadded disease of malignant endocarditis. Further research is evidently called for.

**ACTION OF  
STREPTO-  
COCCUS  
FÆCALIS.**

In a preliminary report on the action of streptococcus fæcalis and of its chemical products, Dr. Sidney Martin discusses the importance of the investigation of this micro-organism, which exists normally in the fæces of the human being (and other animals) and which is capable of producing in man the lesions of cystitis, otitis media, septicæmia, and infective (malignant) endocarditis. The streptococcus is readily cultivated and is a micro-organism of some vitality, since it may be grown for long

periods, apparently without any loss of cultural or pathogenic properties. As regards its action in animals, no pathogenic effect was produced in mice by subcutaneous injection; but in rabbits by intravenous injection, a pronounced effect was produced on the body temperature and on nutrition, the condition ending fatally, and the post-mortem in the single experiment performed showing a small vegetative growth on the mitral valve containing the streptococcus. As regards the toxin of the micro-organism, one experiment seemed to show that the main poisonous product existed in the bodies of the streptococcus (an endotoxin), this poison killing a rabbit in the dose (approximately) of 0.05 gramme per kilo. of body weight.

MEDICAL  
OFFICER'S  
REPORT.

I have the honour to be,

Sir,

Your Obedient Servant,

ARTHUR NEWSHOLME.

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## APPENDIX A.

## No. 1.

\* SUMMARY of the WORK of the MEDICAL INSPECTORS  
during the YEAR 1906.

## A.—DISEASE AND SANITARY ADMINISTRATION.

The following sanitary districts were visited by the Medical Inspectors with special reference to outbreaks of infectious disease of one and another sort, and to general sanitary circumstances and administration, viz. :—

Name of District.	Nature of Inquiry.
††Aberayron, R.D. ... ..	Sanitary state and administration.
†Aberayron, U.D. ... ..	Do. do.
†Aberdare, U.D. ... ..	Infectious disease; sanitary state and administration.
†Abersychan, U.D. ... ..	Enteric fever; sanitary state.
†Ashton-under-Lyne, U.D. ... ..	Sanitary state and administration (small-pox).
†Audenshaw, U.D. ... ..	Sanitary state and administration.
††Batley, U.D. ... ..	Do. do.
†Belmont Asylum (Sutton) ... ..	Enteric fever in the Asylum.
††Bourne, R.D. ... ..	Scarlatina and diphtheria; sanitary state and administration.
†Braintree, R.D. ... ..	Infectious disease and sanitary state of Coggeshall; sanitary administration in the Rural District.
†Brandon and Byshottles, U.D. ... ..	Enteric fever; sanitary state and administration.
†Brecknock, R.D. ... ..	Sanitary state and administration.
†Bridport, R.D. ... ..	Do. do.
Bromley, R.D. ... ..	Lodging accommodation of fruit and hop-pickers.
††Chester-le-Street, R.D. ... ..	Sanitary state and administration.
Chippenharn, R.D. ... ..	Drainage, &c. of Corsham; sanitary administration.
†Darlaston, U.D. ... ..	Sanitary state and administration.
†Denton, U.D. ... ..	Do. do.
†Droylsden, U.D. ... ..	Do. do.
†Eaton Socon, R.D. ... ..	Do. do.
††Ebbw Vale, U.D. ... ..	Infectious diseases; sanitary state and administration.
†Failsworth, U.D. ... ..	Sanitary state and administration.
†Garstang, R.D. ... ..	Sanitary state of the Parish of Hambleton.
†Hambleton, R.D. ... ..	Sanitary state and administration.
†Haverfordwest, U.D. ... ..	Enteric fever; sanitary state and administration.
†Hurst, U.D. ... ..	Sanitary state and administration.
†Lees, U.D. ... ..	Do. do.
†Limehurst, R.D. ... ..	Do. do.
†Mansfield, U.D. ... ..	Enteric fever.
†Market Harborough, R.D. ... ..	Diphtheria at Fleckney.
†Mitford and Launditch, R.D. ... ..	Diphtheria in the Parish of Guist; sanitary state and administration.

\* Throughout this summary the following abbreviations are used:—U.D. = a Borough or Urban District; R.D. = Rural District; P.S.D. = Port Sanitary District; Jt.H.D. = A Joint District for hospital purposes, formed under the Public Health Act, 1875, or the Isolation Hospitals Acts, 1893 and 1901.

† See Appendix A, No. 2.

‡ Extracts from the reports on these districts are reproduced in this volume, see Appendix A, No. 3.

A.—DISEASE AND SANITARY ADMINISTRATION—*continued.*

Name of District.	Nature of Inquiry.
†Mossley, U.D. ... ..	Sanitary state and administration.
†New Quay (Cardigan), U.D. ... ..	Do. do.
Oldham, U.D. ... ..	Sanitary administration with reference to small-pox.
Ongar, R.D. ....	Lodging accommodation of pea-pickers.
†Panteg, U.D. ... ..	Enteric fever; sanitary state.
Petersfield, R.D. ... ..	Enteric fever in Bedales School, Steep.
†Pontypool, U.D. ... ..	Enteric fever; sanitary state.
†Saffron Walden, R.D. ... ..	Sanitary state and administration of Great Chesterford Village.
†Sheerness, U.D. ... ..	Brief re-inspection as to enteric fever, sewage disposal, water supply, &c.
Southport, U.D. ... ..	Sanitary administration.
†Southwick-on-Wear, U.D. ... ..	Zymotic diseases; sanitary state and administration.
†St. Neots, R.D. ... ..	Sanitary state and administration.
†Tavistock, R.D. ... ..	Diphtheria at Princetown; sanitary state and administration.
†Tiverton, R.D. ... ..	Diphtheria at Bradninch.
††Trowbridge, U.D. ... ..	Sanitary state and administration; appointment of Inspector of Nuisances.
Upton-on-Severn, R.D. ... ..	Lodging accommodation for fruit and hop-pickers.
†Walmer, U.D. ... ..	Sanitary state and administration.
Wandsworth Prison ... ..	Enteric fever in the prison.
††Whickham, U.D. ... ..	Housing accommodation; back-to-back houses at Marley Hill; sanitary state and administration.
††Wigan, U.D. ... ..	Infantile mortality; enteric fever and diarrhoea; sanitary state and administration.
†Windsor, R.D. ... ..	Need for a drainage system in Parishes of Sunninghill and Sunningdale; sanitary state and administration.

† See Appendix A, No. 2.

†† Extracts from the reports on these districts are reproduced in this volume, see Appendix A, No. 3.

## B.—HOSPITALS, &amp;c.

Local inquiries were held by the Medical Inspectors with reference to the provision of hospital accommodation for the isolation of cases of infectious disease in connection with the following districts :—

Acton, U.D.	Congleton and District,
Aston Manor, U.D.	Jt.H.D.
Bangor, U.D.	Conway, U.D.
Barking Town, U.D.	Derwent Valley, Jt. Small-
Barry, U.D.	pox, H.D.
Bradford (Yorks), U.D.	Dewsbury, Jt.H.D.
Brandon and Byshottles,	Gelligaer and Rhigos, R.D.
U.D., and Durham, R.D.	Greenford, U.D.
Bury and District, Jt.H.D.	Hampton, U.D.
Cheshunt, U.D.	Harrogate and Knaresborough,
Colchester, U.D.	Jt.H.D.

**B.—HOSPITALS, &c.—continued.**

Hemel Hempstead, R.D. and U.D.	Penrith, U.D.
Hove, U.D.	Rhyl, U.D.
Leek, U.D.	River Blyth Port.
Lincoln, U.D.	Royston, Ashwell and Melbourne, Jt.H.D.
Llantrisant and Llantwit Vardre, R.D.	Settle, R.D.
Loughborough, U.D.	Sevenoaks, R.D.
Manchester, U.D. (2).	South Rotherham, Handsworth and Kiveton Park, Jt.H.D.
Middlesex, Small - pox, Jt.H.D.	South Staffs, Small-pox, Jt.H.D.
North Derbyshire, Jt.H.D.	Wolverhampton, U.D.
Orsett, Jt.H.D.	

Inquiries were held as to the provision of a public mortuary for Birmingham, U.D.; of a mortuary, Coroner's Court, post-mortem room, disinfecting station and shelter for Deptford, U.D.; of a disinfecting station for Kensington, U.D.; and of a steam laundry at the hospital for Portsmouth, U.D.

**C.—SEWERAGE AND SEWAGE DISPOSAL.**

Inquiries in this connection were held as follows :—

Great Crosby, U.D.	Okehampton, R.D. (Exbourne).
Greenford, U.D.	Orsett, R.D.
Manchester, U.D.	

**D.—WATER SUPPLY.**

Inquiries were held into the water supply of the following districts :—

Beverley, U.D.	Bridlington, R.D. (Reighton).
Bicester, U.D.	

**E.—BYELAWS.**

Inquiries with reference to byelaws were held as follows :—

Atherstone, R.D.	Islington, U.D.
Bootle, U.D.	Liverpool, U.D.
Guildford, R.D.	Midhurst, R.D.
Hanley, U.D.	Shoeburyness,

**F.—SCAVENGING.**

Under this head inquiry was made at :—

Bridlington, R.D. (Hunmanby).

## G.—VACCINATION INSPECTION.

In addition to the routine inspection of vaccination in England and Wales (page x), special visits by Medical Inspectors with reference to vaccination administration were made to the Unions mentioned below. The visit to the Union indicated in italics was made owing to the prevalence of small-pox in the Union :—

Barnsley.	Lambeth. (2.)
Blaby.	Maldon.
Burnley.	Newport (Mon.).
Burton-on-Trent.	<i>Oldham.</i>
Cricklade and Wootton Bassett.	Stepney.
Derby.	Trowbridge and Melksham.
Dore.	Wandsworth.
Gloucester.	Wellingborough.
Halifax.	Whitehaven.

## H.—CONFERENCES WITH LOCAL OFFICIALS.

Conferences on sanitary matters were held locally with various officials of the following districts :—

Aldershot, U.D.	St. Just, U.D.
Clayton-le-Moors, U.D.	St. Neots, R.D. and Eaton
Compstall, U.D.	Socon, R.D.
Farnham, R.D. and U.D.	Southam, R.D.
Fleet, U.D.	Tottenham, U.D.
Holderness.	Walton-on-Thames, U.D.
Poole, U.D. (Branksome).	Wrexham, U.D.

## I.—MISCELLANEOUS.

Dr. Bulstrode continued further his general inquiries into sanatoria for consumptives, and held an inquiry into an application by the Sandgate U.D. for sanction to Hospital Regulations.

Dr. Farrar held an inquiry into an application by the Basingstoke U.D. for a loan to defray the expenses caused by an epidemic of enteric fever; he also visited Hertford R.D. to inspect a shelter for vagrants on a farm at Knebworth, and he made a general inquiry into the lodging and accommodation of hop-pickers and pickers of fruit and vegetables.

Dr. Johnstone informally reported upon an outbreak of small-pox in Bridlington U.D.

Dr. Darra Mair took part in an inquiry into an application under section 10 of the Housing of the Working Classes Act, 1890, as to Warner Street (Italian Colony) area by the Holborn U.D. and began an investigation of the advantages and disadvantages of Intercepting Traps.

I.—MISCELLANEOUS—*continued.*

Dr. Manby visited Middlewich U.D. and Wath-upon-Dearne U.D. in connexion with Dairies, Cowsheds and Milk-shops Regulations.

Dr. Reece held an inquiry into an allegation against the Medical Officer of Health in Pontardawe R.D.; accompanied by Mr. G. C. Hancock he held conferences locally as to the provision of a mooring place for vessels over 130 feet in length in Teignmouth Port, and he held an inquiry on an application by Manchester P.S.A. for a Provisional Order giving them additional powers under sections 116–119 of the Public Health Act, 1875, *re* Unsound Food.

Dr. Deane Sweeting visited Edinburgh with reference to the appointment of a new Teacher of Vaccination, and he also visited Galway with reference to the appointment of a Teacher of Vaccination in Queen's College.

Dr. Wheaton visited Wrexham U.D. with reference to the growth of strawberries on a sewage farm. He also completed an inspection of the River Tweed with reference to an application for the issue of a Provisional Order constituting a Special Joint Committee under the Rivers Pollution Prevention Act, and the Border Councils Act, of 1898.

## K.—INQUIRIES BY THE ASSISTANT INSPECTOR IN THE MEDICAL DEPARTMENT.

Mr. Huddart inquired into the arrangements for the performance of the duties of nuisance inspector in the following districts :—

Blything, R.D.	St. Neots, R.D.
Brampton and Walton, U.D.	Swavesey, R.D.
Bridport, R.D.	Thingoe, R.D.
Chepping Wycombe, U.D.	Tutbury, R.D.
Eaton Socon, R.D.	Wantage, R.D.
New Mill, U.D.	Wath, R.D.
St. Ives, R.D.	Witney, R.D.
St. Ives, U.D.	

Mr. Huddart also visited the Unions named below as to the administration of the Vaccination Acts, and especially as to the performance of duty by the Vaccination Officers :—

Ashton-under-Lyne.	Bridgwater.
Axminster.	Bridport.
Barrow-on-Soar.	Brixworth.
Bellingham.	Bury.
Bideford.	Calne.
Bourne.	Colchester.
Brackley.	Dunmow.
Bradford-on-Avon.	East and West Flegg.
Braintree.	Faringdon.

K.—INQUIRIES BY ASSISTANT INSPECTOR—*continued.*

Gateshead.	Nuneaton.
Guisborough.	Pershore.
Headington.	Peterborough.
Hexham.	Plomesgate.
Holsworthy.	Poole (2).
Horsham.	Prestwich.
Howden.	Reading.
Lampeter.	Ruthin.
Lexden and Winstree.	Samford.
Lichfield.	Scarborough.
Lincoln.	Sherborne.
Maidenhead.	Solihull.
Malmesbury.	Swansea.
Mere.	Tendring.
Midhurst.	Trowbridge and Melksham.
Middlesbrough.	Tynemouth.
Newcastle Emlyn.	Whitchurch (Salop).
Newent.	Wight, Isle of.
Newport (Mon.).	Witney.
Northampton.	Wokingham.
North Bierley.	Wolverhampton.

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## APPENDIX A., No. 2.

ABSTRACT of MEDICAL INSPECTIONS made in 1906 with regard to the INCIDENCE of DISEASE on particular places, and to questions concerning LOCAL SANITARY ADMINISTRATION.

[Where price is given the report can be obtained either direct or through any bookseller from Messrs. Wyman & Son, 109, Fetter Lane, Fleet Street, E.C. Extracts from the reports relating to districts indicated by an asterisk are reproduced in this volume, see App. A, No. 3.]

1.\***ABERAYRON RURAL DISTRICT (CARDIGANSHIRE)**; population (1901), 8,170; Dr. Fletcher. [No. 283, Price 5d.]

*Authority concerned* : Aberayron Rural District Council.

*Ground of Inquiry* : Persistence on the part of the Council in appointing two Medical Officers of Health against the Board's recommendation to appoint only one for the whole district.

*Chief Facts reported by Inspector* : General want of supervision throughout the district. Water supplies unsatisfactory. Drainage for liquid house refuse deficient, and where provided, often defective. Many excremental nuisances, and very many cottages unprovided with closet accommodation. Not a few of the dwellings damp and insanitary owing to dilapidation, to defective structure, or to both causes. Slaughter-houses not properly supervised, not always suitably constructed, and not always kept clean. No register kept of cowkeepers and purveyors of milk. Cowsheds not up to the standard of modern requirements. No regulations under the Dairies, Cowsheds and Milkshops Order, 1885, in force. None of the voluntary Acts have been adopted. No action under the Public Health (Water) Act, 1878. No bye-laws in force. No isolation hospital. Grossly inadequate salaries of £20 per annum paid to each of the two Medical Officers of Health, and a paltry salary of £30 per annum to the Inspector of Nuisances, an aged man who is also master of the workhouse, and who has had no training in the duties of the former office.

2. **ABERAYRON URBAN DISTRICT (CARDIGANSHIRE)**; population (1901), 1331; Dr. Fletcher. [No. 283, Price 5d.]

*Authority concerned* : Aberayron Urban District Council.

*Ground of Inquiry* : The district forms part of the Aberayron Registration District, together with Aberayron Rural and New Quay Urban Districts, and inspection of Aberayron and New Quay Urban Districts was ordered in conjunction with inspection of Aberayron Rural District.

*Chief Facts reported by Inspector* : Water supplies unsatisfactory, and much waste of money on abortive schemes

for public water service. Sewerage elementary in character and defective. No common outfall, and no sewage treatment. House drainage deficient, and where provided, often defective. Closet accommodation generally defective, and in some instances non-existent. A number of dwellings quite unfit for human habitation. Slaughter-houses very unsatisfactory except in one instance. No register kept of cowkeepers and purveyors of milk, most of which is brought in from the Rural District. Cowsheds at Pen Garreg, a large dairy farm, fairly satisfactory, dairy excellent and well kept. No Regulations under Dairies Cowsheds and Milkshops Order. None of the Voluntary Acts adopted. Bye-laws in force as to nuisances, pleasure boats and vessels, slaughter-houses, and bathing. No isolation hospital. Salary of Medical Officer of Health, £20 per annum; salary of Inspector of Nuisances, £20 per annum.

3. ABERDARE URBAN DISTRICT (GLAMORGAN); population (1901), 43,365; Dr. Spencer Low. [No. 259, Price 8d.]

*Authority concerned*: Aberdare Urban District Council.

*Ground of Inquiry*: High mortality from various infectious diseases. Absence of annual reports from Medical Officer of Health, who was not appointed under the Board's Order. Application for sanction to increase of salary of Inspector of Nuisances.

*Chief Facts reported by Inspector*: Many houses unfit for human habitation. Defective methods of refuse disposal. Some cowsheds and common lodging houses unsatisfactory, and some hand-flushed waterclosets. Disinclination of Council to deal with some matters of prime importance, such as provision of a proper infectious diseases hospital and of a destructor. No systematic bacteriological examination of material in doubtful cases of infectious disease. High general death-rate, zymotic death-rate, and infantile mortality into the latter of which insufficient investigation has hitherto been made. Appointment of a whole-time Medical Officer of Health which had been decided upon before inspection, is calculated to lead to the adoption of improved methods of administration. Work of Inspectors of Nuisances well performed.

4. ABERSYCHAN URBAN DISTRICT (MONMOUTHSHIRE); population (1901), 17,768. Dr. Johnstone. [No. 276, Price 8d.].

*Authority concerned*: Abersychan Urban District Council.

*Ground of Inquiry*: Undue prevalence of enteric fever in 1906.

*Chief Facts reported by Inspector*: During the period June 23rd to November 10th, 1906, 55 cases of enteric fever were reported in 37 houses. The houses invaded were mainly in the neighbourhood of Pontypool, where an outbreak of enteric fever was in progress. Eight cases were connected with polluted wells. Personal infection either from cases in Pontypool or from other cases in Abersychan is held responsible for most of the remaining

attacks. Isolation hospital—a temporary building with a bad approach and water supply inadequate in case of fire. No disinfecter provided. Crowding of dwellings on area in parts of the district; lack of sufficient air-space around dwellings in other parts. Intermittent water supply in dry weather, and hence insufficient flushing of water closets. Sewage discharged into the river untreated.

5. ASHTON-UNDER-LYNE BOROUGH (LANCASHIRE); population (1901), 43,890; Dr. Fletcher. [No. 271, Price 6d.]

*Authority concerned:* Ashton-under-Lyne Town Council.

*Ground of Inquiry:* Late Medical Officer of Health, deceased, received a salary of £130 per annum. Appointment of new Medical Officer of Health, in general practice, without D.P.H. and without training or experience, at a salary of £80 per annum in opposition to the Board's expressed views.

*Chief Facts reported by Inspector:* Water-supply good and ample in conjunction with Urban Districts of Stalybridge, Dukinfield, Hurst, Mossley and Audenshaw, and Limehurst Rural District—moorland water. Sewerage apparently satisfactory. Sewage disposal works on an extensive scale, estimated cost £88,000, have been provided. Occasional nuisance alleged to arise therefrom. Conversion of privy-middens to waste water-closets, on an idea to change the privy-middens to a water-carriage system, has been very largely secured, but the work is not completed. House drainage, generally, satisfactory, many old and dilapidated houses, but house accommodation on the whole fairly satisfactory. Slaughter-houses mostly exhibit defects—a public abattoir desirable. Some of the cowsheds are old and unsatisfactory. The isolation hospital is inadequate and of poor structure—discreditable to a town of nearly 50,000 inhabitants—and is seldom used for ordinary infectious disease; has been used for small-pox. A small-pox hospital has lately been provided jointly with Stalybridge, Hurst and Audenshaw Urban and Limehurst Rural Districts. Much good work has been done by the town council. For a town of this size a properly trained, experienced and qualified Medical Officer of Health is required, prepared to devote most, if not all, of his time to his public health duties.

6. AUDENSHAW URBAN DISTRICT (LANCS.); population (1901), 7,216; Dr. Fletcher. [No. 270, Price 3d.]

*Authority concerned:* Audenshaw Urban District Council.

*Ground of Inquiry:* Inspection in common with a group of eight other neighbouring Lancashire districts under part-time Medical Officers of Health, none of whom were qualified in Public Health, and five of whom held their appointments without the Board's sanction, with a view to repayment by the County Council of moiety of their salaries.

*Chief Facts reported by Inspector:* Water supply, with the exception of about a dozen houses supplied by the Corporation of Manchester, is from the Ashton-under-Lyne,

Stalybridge, and Dukinfield (District) Joint Waterworks. Sewage on the whole satisfactory, considerable lengths of new sewers laid during recent years, but some old rubble sewers still exist. Sewage-disposal works jointly with Stalybridge and Dukinfield. Certain houses, representing a population of some 500 persons, drain into the Manchester sewers. Houses generally are provided with drains, but these are in some instances defective. House accommodation generally fair, but some old property exists. Closet accommodation usually of the privy midden type, but conversion to waste-water-closets is proceeding. Slaughter-houses are not altogether satisfactory. Cowsheds in some cases are well constructed, but in others are old and ill-ventilated; no register of cowkeepers and purveyors of milk is kept. Regulations as to dairies, cowsheds and milk-shops in force, but require amendment. No isolation hospital for ordinary infectious disease. Small-pox hospital jointly with Ashton-under-Lyne, Stalybridge, and Hurst Urban, and Limehurst Rural Districts. Salary of Medical Officer of Health only £20 per annum; he holds no diploma in Public Health, and had no experience before he was appointed.

- 7.\*BATLEY BOROUGH (YORKSHIRE); population (1901), 30,321; Dr. Spencer Low. [No. 242, Price, 9d.]

*Authority concerned:* Batley Town Council.

*Ground of Inquiry:* Persistently high general and zymotic death rates, and excess of infantile mortality; Registrar-General's return for September quarter, 1905, showing serious mortality from diarrhoea.

*Chief Facts reported by Inspector:* Excrement and refuse commonly disposed by privy middens; middens emptied by contents being thrown out on to yard, thereby polluting its surface. Water carriage system of excrement disposal discouraged, probably owing to insufficiency of water for flushing purposes.

Hospital accommodation provided only for cases of small-pox, on site in undue proximity to houses. Prevalence of enteric fever probably due to absence of hospital isolation and to addition of infectious discharges to middens. Methods of disinfection deficient.

Spread of scarlatina associated with absence of hospital isolation.

There would be advantage in combination of this and neighbouring districts for purpose of appointing a Medical Officer of Health to devote his whole time to public health duties.

Privy midden system should be abandoned and sufficient accommodation should be provided in hospital for cases of infectious disease.

8. BELMONT ASYLUM FOR IMBECILES, SUTTON, SURREY; Dr. Copeman. [No. 248, Price 5d.]

*Authorities concerned:* Metropolitan Asylums Board and the Sutton Urban District Council.

*Ground of Inquiry:* Request from Sutton Urban District Council for official enquiry into causation of enteric fever at Belmont Asylum.

*Chief Facts reported by Inspector:* During the months of May, June and July, 1906, an outbreak of enteric fever, causing 64 cases and 11 deaths, occurred among the inmates of the Belmont Asylum; the diagnosis of which was, however, for some time in doubt owing to anomalous nature of symptoms in the first cases. Under the circumstances, requests for assistance from this Board were received from the Metropolitan Asylums Board and the Sutton Urban District Council. Post-mortem examination of patients dying of pulmonary and other complaints as well as from the disease in question revealed in certain instances definite evidence, in the intestines, of more or less recent enteric fever, while information as to presence of this disease was also afforded by examination of the blood of a considerable number of the inmates, some of whom had not exhibited any recognisable symptoms clinically.

Careful investigation of sewerage and drainage, water-supply, milk and other foodstuffs, farming operations on old irrigation areas, and sources from which mattresses and bedding were obtained having thrown no light on the origin of infection, the conclusion was arrived at that this was probably due to the presence of one or more "typhoid-carriers" among patients who had been transferred to Belmont from other Institutions; subsequent spread of the disease in the Asylum being due, in some degree, to the filthy and degraded habits of the inmates.

9.\*BOURNE RURAL DISTRICT (LINCS., KESTEVEN); population (1901), 13,212; Dr. Mivart. [No. 247, Price 4d.]

*Authority concerned:* Bourne Rural District Council.

*Ground of Inquiry:* Unwholesome conditions reported in various localities—prevalence of scarlatina and diphtheria.

*Chief Facts reported by Inspector:* Dilapidated and unwholesome dwellings found generally. No paving round houses, gutter spouting and down pipes absent or defective. Houses scarce and costly. More houses for working people needed. Water supplies generally extremely defective and scarce in many places, although water supply for most part of district could be obtained if sought. Midden or vault privies almost universal. Many filth nuisances. Most villages provided with some sort of sewerage or drainage. But sewers and drains defective and discharging into ditches or watercourses, causing constant nuisance.

Forty-one cases of scarlatina at Castle Bytham (population, 618) in 1905. Insufficient measures of disinfection and precaution. Disease now abated. 15 cases of diphtheria at Pointon (population, 405) in 1905. Mortality 40 per cent. Spread by personal communication, and probably through undetected cases. Disease now abated. No proper hospital. No disinfecting apparatus of any

service. At Castle Bytham an unventilated wooden shed, without drainage or water supply, used to house scarlatina patients and uninfected relatives all together. Slaughter-houses not regulated. Dairies, Cowsheds and Milk-shops Orders not enforced. Many serious filth nuisances detected upon and in connection with milk dealers' premises. Inspector of Nuisances insufficiently paid and required to carry out certain duties of Surveyor. No adoptive Acts in force. Rural District Council delegate to Parochial Committees such powers as they are able to confer. District generally backward.

10. BRADNINCH, A TOWN OF 1,000 INHABITANTS COMPRISED WITHIN THE TIVERTON RURAL DISTRICT; population (1901), 15,339; Dr. Manby.

*Authority concerned*: Tiverton Rural District Council.

*Ground of Inquiry*: Continued prevalence of diphtheria; Rural District Council asked for inquiry.

*Chief Facts reported by Inspector*: 48 cases of diphtheria with six deaths in Bradninch between September 22nd, 1905, and September 15th, 1906; four cases of suspicious illness (three deaths) in late September and early October, 1905, not regarded as diphtheria by *locum tenens*. Diphtheria patients were largely children attending the public elementary schools, and speaking generally there was much evidence of conveyance of infection from person to person; origin of first case not discovered.

Steps taken by Rural District Council to cope with outbreak prior to Inspector's visit; school closure on several occasions; isolation of some of the cases clinically diphtheria. Subsequently the throats of all school children were frequently examined, and swabs were taken for bacteriological examination. All cases bacteriologically diphtheria were isolated in hospital; these included several "carrier cases" who had previously had diphtheria but were, in September, 1906, clinically in good health. Prophylactic injections of anti-toxin, and throat washes, were provided free of charge to all desiring them. Drainage system of Bradninch defective. Water supply not sufficient in quantity. No regulations in force as to dairies, cowsheds and milkshops.

11. BRAINTREE RURAL DISTRICT (ESSEX); population (1901), 18,109; Dr. Fletcher. (Previously inspected and reported upon by Dr. Fletcher in 1899.) [No. 244, Price 6d.]

*Authority concerned*: Braintree Rural District Council.

*Grounds of Inquiry*: Continued complaints of nuisance from drainage of village of Coggeshall, and of prevalence of diphtheria in very fatal form.

*Chief Facts reported by Inspector*: The report deals primarily with Coggeshall, but also relates to a re-survey of the district in connection with the administration, the arrangements not being such as the Board desired, especially as to appointment of Inspectors of Nuisances. State of Coggeshall as to water supply and drainage very

bad. Serious pollution of river with sewage. Facts as to prevalence of diphtheria not so serious as represented to the Board. Eleven cases arose in connection with the British School, and the heavy mortality (eight deaths) gave rise to exaggerated accounts. There was also some prevalence of scarlet fever at Coggeshall, and of diphtheria at Weathersfield. There is an isolation hospital for Brain-tree Rural and Urban Districts, and also a joint small-pox hospital. Administration of the district generally is lax, and very little advance has been made during the past seven years. Water supplies, excrement disposal, and sewerage and house-drainage require more attention. A good whole-time Inspector of Nuisances is necessary, and combination with other districts for the appointment jointly of a properly qualified and experienced Medical Officer of Health, devoting all his time to his public health duties is very desirable.

12. BRANDON AND BYSHOTTLES URBAN DISTRICT (DURHAM); population (1901), 15,573; Dr. Fletcher. [No. 260, Price 1s.]

*Authority concerned:* Brandon and Byshottles Urban District Council.

*Ground of Inquiry:* Prevalence of enteric fever, especially during 1904 and 1905.

*Chief Facts reported by Inspector:* Water supply, excepting some farms and outlying houses, from the Weardale and Consett Water Company. District mainly a colliery and agricultural one. Pit villages mostly sewered, and sewage treated at a number of outfall works of varying types. House drainage largely by open channels. Excrement disposal mostly by privy-middens, many open, and many in disrepair. At Brandon Colliery many ash-closets recently constructed on County Council's plan. House accommodation in pit villages unsatisfactory, and much overcrowding present. Slaughter-houses fairly satisfactory. Register of Cowkeepers and Purveyors of Milk not kept up to date. Some of cowsheds very inferior. Regulations as to Dairies, Cowsheds and Milk-shops in force. Isolation Hospital provided for ordinary infectious diseases, and district recently combined with Durham Rural District for Small-pox Hospital by County Council Order. Enteric fever. In 1904, 80 cases; in 1905, 102 cases; in 1906, 48 cases. No definite cause discovered, but suspicion that initial cases were those of men and boys working, and infected, in the pits, and later cases females who had acted as nurses. This theory worked out. Apparently considerable spread from house to house. Brandon Colliery especially affected.

13. BRECKNOOK RURAL DISTRICT (BRECON); population (1901), 9,758; Dr. Manby. [No. 258, Price 4d.]

*Authority concerned:* Brecknock Rural District Council.

*Grounds of Inquiry:* Multiple and inadequately paid Medical Officers of Health. Also to ascertain sanitary

progress made in district since inspection by Dr. Fletcher in 1895.

*Chief Facts reported by Inspector:* Three Medical Officers of Health, each paid £20 per annum. Area of the smallest of the three districts 75 square miles. District Council apparently not greatly interested in sanitary condition of their district. The Medical Officers do not attend meetings of the Rural District Council, nor do they submit reports nor keep journals. Little done by these officers in the way of systematic inspection. Some improvement in the sanitary condition of the district since 1895. Improved water supply urgently needed at Trecastle. Llangorse, Talybont, Trecastle and other places require drainage, and generally throughout the district the conversion of privies into water-closets or earth-closets should be hastened. Also proper closet accommodation for dwellings at present wanting such. Need for systematic enforcing of the Dairies, Cowsheds and Milk-shops Order and the Regulations made thereunder. Rural District Council advised to combine, with Borough of Brecon or otherwise, for the provision of adequate isolation hospital accommodation, and for a steam disinfecter. Rural District Council further advised to appoint one Medical Officer of Health at adequate salary, and to require him to devote a definite amount of time per week to his duties.

14. BRIDPORT RURAL DISTRICT (DORSET); population (1901), 6,998; Dr. Spencer Low. [No. 249, Price 3d.]

*Authority concerned:* Bridport Rural District Council.

*Ground of Inquiry:* Inefficient manner in which duties of nuisance inspection has been performed, and unsatisfactory sanitary administration of district.

*Chief Facts reported by Inspector:* Persistent neglect of work by Inspector of Nuisances, in spite of promises of amendment. Nuisances prevalent in the district, especially those arising from defective house-drainage. System of sewerage and sewage disposal inadequate at Charmouth. Persistence of scarlatina in Charmouth, probably associated with "missed" or concealed cases.

Persons or trades requiring registration not registered.

- 15.\*CHESTER-LE-STREET RURAL DISTRICT (DURHAM); population (1901), 60,552; Dr. Fletcher. [No. 250, Price 8d.]

*Authority concerned:* Chester-le-Street Rural District Council.

*Ground of Inquiry:* Representations by the County Council of insanitary conditions in Usworth, and that the Public Health Act, 1875, and the Housing of the Working Classes Act had not been properly put in force.

*Chief Facts reported by Inspector:* Water supply almost entirely by public service from three different water companies and one colliery company. Pit villages sewered, and sewage treated at various outfall works in the majority of instances. Over 55 miles of sewers laid

since the middle of 1897—*i.e.*, in nine years. Water-closets in use here and there, but excrement disposal mostly by privy-ashpits or “ash-closets.” During recent years many hundreds of privy-ashpits replaced by ash-closets on County Council design. House accommodation inadequate to meet requirements of the large population. Many old insanitary dwellings, and a vast amount of overcrowding. Efforts being made to remedy this serious condition, but District Council will not adopt Part III. of Housing of Working Classes Act, 1890. This is the most serious defect in the circumstances and sanitary administration of the district. There are two good hospitals, one for ordinary isolation, and one for isolation of small-pox. A nominally part-time Medical Officer of Health, holding a D.P.H., at an annual salary of £245, devotes about three-quarters of his time to public health work, and has under him three certificated Inspectors of Nuisances who receive salaries of £120, £130, and £120 per annum. Types of colliery dwellings illustrated in Appendix.

**16. DARLASTON URBAN DISTRICT (STAFFORDSHIRE);** population (1901), 15,395; Dr. Farrar. [No. 243, Price 4d.]

*Authority concerned:* Darlaston Urban District Council.

*Grounds of Inquiry:* Considerable mortality from zymotic diseases, and high general death-rate and infantile death-rate in the district.

*Chief Facts reported by Inspector:* Water supply from South Staffordshire Water Company; satisfactory. Sewerage satisfactory except in respect of Moxley. There are about 800 water-closets in the district, but more than 70 per cent. of the houses have privy-middens of a very objectionable type, which prevents the due admixture of dry ashes with excremental matter, and favours the accumulation of liquid sewage. Scavenging generally neglected. Housing conditions are particularly bad in this district. About 500 good artisans' dwellings have been erected in the last ten years, but more than two-thirds of the houses are jerry-built and in very bad repair. Some particular instances of houses in very bad repair are mentioned.

Defective sanitary conditions no doubt contribute largely to the high general, infantile and zymotic death-rates of the district; the undesirable methods of excrement and refuse disposal tend to increase the death-rate from diarrhoea and enteritis, while defective housing conditions and overcrowding tend to promote phthisis and similar disorders. Maternal ignorance and the large employment of women in factories, together with the notoriously large consumption of alcohol, are probably important factors in the high infantile death-rate.

The Sanitary Inspector is old and incompetent to the duties of his post. The sanitary requirements of the district call for the appointment of a qualified sanitary inspector in full bodily vigour. There is great need for

intelligent and systematic inspection of houses, the provision of new artisans' dwellings is urgently required. The methods of excrement disposal call for special attention. A suitable disinfectant is needed, and the provision of a destructor is recommended.

17. DENTON URBAN DISTRICT (LANCASHIRE); population (1901), 14,934; Dr. Spencer Low. [No. 264, Price 3d.]

*Authority concerned:* Denton Urban District Council.

*Ground of Inquiry:* District comprises one of a populous group in which sanitary administration appeared in some respects to be defective.

*Chief Facts reported by Inspector:* Insufficient supervision has been exercised over insanitary house property. There is considerable amount of slum property in district where nuisances abound. Midden-privies numerous. Nuisance at times from refuse tips. Considerable amount of sanitary improvement has been effected in recent years, but much remains to be done. Arrangements for isolation of infectious cases insufficient, and no steam disinfectant available.

Cause of high infantile mortality requires investigation. Performance of duties by Medical Officer of Health perfunctory in several respects. Separation of duties of nuisance inspection and those of Surveyor highly desirable as present officer cannot adequately perform those of both offices.

Necessity exists for appointment of whole-time Medical Officer of Health who could conveniently hold office also in adjoining districts.

18. DROYLSDEN URBAN DISTRICT (LANCASHIRE); population (1901), 11,087; Dr. Spencer Low. [No. 263, Price 3d.]

*Authority concerned:* Droylsden Urban District Council.

*Ground of Inquiry:* District is one of a populous group in which sanitary administration appeared in some respects to be defective.

*Chief Facts reported by Inspector:* Considerable amount of slum property, where nuisances abound. Some of the old sewers defective, and the sewage outfall works discharge an offensive effluent; these works were about to be remodelled at the time of inspection. Large number of offensive midden-privies in district. Nuisance occasioned from tipping of house refuse.

Little sanitary activity has been exhibited in Droylsden until the last few years.

Good advice has been given to Council by Medical Officer of Health. The Inspector of Nuisances is also Surveyor, and cannot adequately perform the duties of both offices. Whole-time Inspector required.

Arrangements for isolation of infectious cases insufficient, and there is no steam disinfectant. Refuse destructor required.

19. EATON SOCON RURAL DISTRICT (BEDFORDSHIRE); population (1901), 3,335; Dr. Sweeting. [No. 253, Price 4d.]

*Authority concerned:* Eaton Socon Rural District Council.

*Ground of Inquiry:* As to the need for a whole-time certificated Inspector of Nuisances for this and St. Neot's Rural Districts.

*Chief Facts reported by Inspector:* Many cottages in bad state of repair; some overcrowding of persons in them. Bad paving of back yards. Part of Eaton Socon village supplied from a private water company, and water said to be good; but other parts of village supplied from shallow wells liable to pollution. Some pollution of water courses. Need for public scavenging in Eaton Socon village. No proper fever hospital provided; nor disinfectant. Systematic inspection of district neglected by Medical Officer of Health, who is allowed nothing for travelling expenses. Inspector of Nuisances holds a plurality of appointments, which prevents him giving adequate attention to his sanitary duties; a whole-time officer urgently required.

- 20.\*EBBW VALE URBAN DISTRICT (MONMOUTHSHIRE); population (1901), 20,994; Dr. Farrar. [No. 255, Price 7d.]

*Authority concerned:* Ebbw Vale Urban District Council.

*Ground of Inquiry:* Undue prevalence during recent years of infectious diseases, particularly of scarlatina and diphtheria.

*Chief Facts reported by Inspector:* Water supply satisfactory; upland surface water from Llangynidr mountain. Western Valleys (Monmouthshire) sewerage scheme being carried out in district; but drainage defective in parts of the district, particularly in Beaufort; house drainage of older houses defective. Excrement disposal principally dry-ash closets; privies defective in some parts of the district; scavenging very much neglected in the Beaufort district (North Ward); satisfactory in North Central and South Central Wards. A considerable amount of squalid and insanitary house property still remains, but a large number of good workmen's houses have recently been erected by building clubs.

Examination of the diphtheria statistics shows that, while cases notified as diphtheria have been unduly prevalent, the case mortality has been remarkably light; the incidence has been heaviest in the Beaufort Ward, in which sanitary conditions are more unsatisfactory than in other parts of the district; but school influence, particularly in the Beaufort schools, appears to have been largely responsible for the spread of infection.

In respect of scarlatina, school influence has also been apparently the principal factor in the spread of infection.

The Medical Officer of Health has failed to show sufficient activity in dealing with infectious disease. He has not made sufficient use of his power to recommend closure of schools, and has not made adequate use

of the isolation hospital for the segregation of early cases of infectious disease.

It is greatly to be desired that the Ebbw Vale Urban District Council should endeavour, by combination with adjoining districts, to secure the services of a properly qualified whole-time Medical Officer of Health.

21. **FAILSWORTH URBAN DISTRICT (LANCASHIRE)**; population (1901), 14,152; Dr. Spencer Low. [No. 266, Price 3d.]  
*Authority concerned*: Failsworth Urban District Council.  
*Ground of Inquiry*: District is one of a populous group in which sanitary administration appeared in some respects to be defective.  
*Chief Facts reported by Inspector*: Some insanitary house property in district, and some areas inhabited by persons of filthy habits. Large number of pail and midden-privies. Some cowsheds in very insanitary condition. Arrangements for isolation of infectious cases insufficient. No steam disinfecter available.  
 There would be advantage in appointment of a whole-time Medical Officer of Health in combination with neighbouring districts.
22. **FLECKNEY (LEICESTERSHIRE)**; population (1901), 1,516; Dr. Darra Mair.  
*Authority concerned*: Market Harborough Rural District Council.  
*Ground of Inquiry*: Prevalence of diphtheria.  
*Chief Facts reported by Inspector*: 160 cases of diphtheria between January and end of July, and 16 deaths. Large increase of cases in June and July. Village sewered in 1893; no public water supply; privy-middens partly; water-closets hand flushed. Soil stiff Boulder Clay. Antitoxin used up to time of visit for treatment of cases of diphtheria, but not prophylactically. This measure recommended: as result, 1,633 throats were examined by Medical Officer of Health and assistants, and antitoxin administered in over 150 cases. Prevalence of diphtheria ceased in September.
23. **GREAT CHESTERFORD VILLAGE (ESSEX)**; population (estimated), 700; Dr. Mivart.  
*Authority concerned*: Saffron Walden Rural District Council.  
*Ground of Inquiry*: Constant complaints from residents regarding bad water supply and many filth nuisances.  
*Chief Facts reported by Inspector*: Village situated upon gravel overlying chalk. Housing of working people fairly satisfactory in character, but limited number of cottages, and cottages really unfit for habitation have therefore to remain inhabited. No public water supply; water exclusively from wells; wholesome water scarce; shallow wells sunk in drift contaminated by soakage; deep wells satisfactory unless contaminated from surface. All wells unprotected. Medical Officer of Health strongly advocates sinking two public tube wells in village at cost of £125. House

drainage faulty or absent. Excrement disposal by unlined cesspit-privies or by privy pails. Garden ground insufficient. Difficulty in disposing of excreta. No public removal of refuse. Scanty gardens already loaded with ashes and refuse, and much complaint as to hardships in the matter.

Outfall of village sewer—consisting of open pipes—into river Cam, above surface of water. Dangerous filth nuisance at this spot, which is frequented by children. There are also untrapped catch-pits in principal street causing offensive nuisance.

Great Chesterford Parish included in Saffron Walden Joint Hospital District. Ten-bed hospital at Saffron Walden. No disinfectors. Dairies, Cowsheds and Milkshops Orders enforced. No regulations any other subject. No Byelaws save as regards "Vans and Tents." No voluntary Act adopted. Village to large extent regulated by Parish Council.

24. **HAMBLEDON RURAL DISTRICT (SURREY)**; population (1901), 21,660; Dr. Thomson. [No. 257, Price 3d.]

*Authority concerned*: Hambledon Rural District Council.

*Ground of Inquiry*: Doubts as to the nature of the local sanitary circumstances and administration.

*Chief Facts reported by Inspector*: Notable change for the better, in the last 20 years, as regards many of the conditions liable to affect the public health; but still need for improvement of dwellings and their surroundings, of many domestic water supplies, of arrangements for the isolation of cases of infectious disease other than small-pox, and of the conditions of dairies and cowsheds.

25. **HAMBLETON TOWNSHIP (LANCASHIRE)**; population (1901), 321; Dr. Reece.

*Authority concerned*: Garstang Rural District Council.

*Ground of Inquiry*: Complaints during the last ten years by certain inhabitants concerning the water supply and want of a sewerage system in the township of Hambleton.

*Chief Facts reported by Inspector*: The village of Hambleton stands on deposited sea sand, and the country is flat. The area of the township is 1,553 acres, the number of houses 81. The assessable value (April, 1906) for special sanitary purposes, £1,680; for poor law purposes, £2,430. A sanitary rate calculated at 1d. in the £ produces £10. The poor rate for the half-year ending March, 1906, was 1s. 6d. The outstanding liabilities are nil.

The water supply is derived from surface wells, constructed of brick and dry steined. Not every house has its own well. The water of many of the wells has been reported unfit for domestic use on chemical analysis, and these wells are still in use. Probably not a single well in the village can be relied on to supply wholesome water. In dry weather many wells fail to yield water. Most houses have storage for rain-water. The demand for a public water supply is not supported by all the villagers.

Slop drainage goes to cesspools, which are seldom emptied, contents soak away or the overflow finds access to ditches, &c.; or the slop water is thrown on the ground. Several farms in the village with undrained farmyards. Roadside ditches black with sewage; in some ditches pipes have been laid and covered over. This procedure has only resulted in the sewage being carried further to pollute the water of ditches from whence cattle drink.

Excrement disposal mainly by tank or vault privies, seldom water-tight, and often placed near wells; contents of these privies generally used in gardens. There is no public scavenging; each householder disposes of his refuse as best he can.

The township urgently requires a public water supply. The water mains of the Fylde Water Board are laid through Garstang Rural District, and a 9-inch main passes within 500 yards of the eastern boundary of Hambleton; from this main the village could be supplied.

The township needs a complete system of sewers. The flat surface of the ground in the district, and the consequent difficulty of disposing of the sewage without raising it by pumping, render the provision of a suitable sewerage system not so simple a matter as the provision of an adequate water supply.

26. HAVERFORDWEST BOROUGH (PEMBROKE); population (1901), 6,007; Mr. Hancock. [No. 239, Price 6d.]

*Authority concerned:* Haverfordwest Town Council.

*Ground of Inquiry:* Recent prevalence of enteric fever.

*Chief Facts reported by Inspector:* Outbreak of enteric fever, comprising 42 cases and four deaths, occurred in the period from 23rd June, 1905, to 10th February, 1906. The outbreak not "explosive" in character; distributed over a wide area; origin of infection obscure; but spread of the fever favoured by the general insanitary condition of the town, and personal infection, the latter owing to want of adequate isolation in the homes of the patients and the absence of an isolation hospital and disinfecting apparatus. In addition, a contaminated milk supply, also suspected as having had concern in the spread of the fever. Milk supplied mainly from farms in the adjoining rural district; few milkshops in the town. Public water supply mainly from three sources, two of which are used for drinking purposes; the third is not intended for such use, as it is known to be polluted with surface drainage, but it is nevertheless used for drinking purposes by the cottagers in its neighbourhood. Public water supply endangered by insanitary conditions at its source and in its distribution, the latter owing to the supply being intermittent, and the use of defective ball hydrants on the mains. A few shallow wells in use, one of which is known to be liable to pollution from surface drainage. Sewers badly ventilated; sewage discharged

into river untreated. Many houses damp and ill-ventilated; some over-crowding. Pigs commonly kept in proximity to dwellings. Some houses entirely undrained; yards and back passages generally ill-paved and ill-drained; many hand-flushed water-closets and midden privies; disposal of the contents of the latter and house refuse unsatisfactory. Results of the work of the Inspector of Nuisances unsatisfactory. Need of the appointment of a qualified Inspector of Nuisances, to make proper systematic inspection of the district.

27. **HURST URBAN DISTRICT (LANCASHIRE)**; population (1901), 7,145; Dr. Spencer Low. [No. 268, Price 3d.]

*Authority concerned*: Hurst Urban District Council.

*Ground of Inquiry*: District is one of a populous group in which sanitary administration appeared in some respects to be defective.

*Chief Facts reported by Inspector*: A number of houses are dilapidated and otherwise insanitary, and some areas are congested. Most closets on the "waste water" system, and pail and midden privies are being replaced by waste water closets. House refuse is carted to tips. Most of the cowsheds are in an unsatisfactory condition.

Sewage disposal, water supply and isolation accommodation for small-pox are arranged for in combination with other districts; there would be advantage in appointing a whole-time Medical Officer of Health in conjunction with other districts.

Isolation accommodation for infectious diseases other than small-pox should be provided, and a steam disinfecter. Systematic house-to-house inspection should be duly carried out.

28. **LEES URBAN DISTRICT (LANCASHIRE)**; population (1901), 3,621; Dr. Spencer Low. [No. 267, Price 4d.]

*Authority concerned*: Lees Urban District Council.

*Ground of Inquiry*: District is one of a populous group in which sanitary administration appeared in some respects to be defective.

*Chief Facts reported by Inspector*: Areas of insanitary house property. Many untrapped slop drains. Excrement commonly disposed of by pail-closets. Closet accommodation very inadequate in some parts of district. Night soil tipped in district, causing nuisance. Slaughter-houses unsatisfactory. Premises in which other regulated trades are carried on are also insanitary.

District Council are much in need of expert guidance, such as would be furnished them by a whole-time Medical Officer of Health appointed in combination with neighbouring districts, and require to devote much more attention to conditions affecting the health of their district.

29. **LIMEHURST RURAL DISTRICT (LANCASHIRE)**; population (1901), 10,338; Dr. Spencer Low. [No. 273, Price 3d.]

*Authority concerned*: Limehurst Rural District Council.

*Ground of Inquiry*: District is one of a populous group in which sanitary administration appeared to be in some respects defective.

*Chief Facts reported by Inspector*: Some houses dilapidated and many have insanitary surroundings, such as overfull ash-pits, obstructed and ill-paved backyards, defective drains, and midden privies in an offensive condition. Improved methods of excrement and refuse disposal are necessary, and hospital accommodation should be provided for infectious diseases other than small-pox. A steam disinfectant is also required.

The condition of most of the cowsheds is very bad.

Modern byelaws should be adopted. The Council should themselves undertake the scavenging of populous places in the district.

The district is in need of the services of a whole-time Medical Officer of Health, who could be best obtained by combination for the purpose with neighbouring districts.

**30. MANSFIELD BOROUGH (NOTTINGHAMSHIRE)**; population (1901), 21,445; Dr. Thomson. [No. 254, Price 4d.]

*Authority concerned*: Mansfield Town Council.

*Ground of Inquiry*: Outbreak of enteric fever.

*Chief Facts reported by Inspector*: During the 17 weeks ending 5th January, 1907, 92 persons in Mansfield Borough were attacked by enteric fever; an unusual number of cases of this fever also occurred during the same period in the adjacent urban district of Mansfield Woodhouse; the fever was uniformly distributed throughout both districts, save for one small area of Mansfield which escaped; dissemination of the disease could not be referred to milk, shell-fish, ice cream, watercress or other green stuff, aerated waters, butter, conservancy conditions, sewerage conditions, or general sanitary circumstances. The public water supply of Mansfield and Mansfield Woodhouse received consideration as a possible agency of the fever, but the evidence available did not warrant either its definite incrimination or its absolute exculpation.

**31. MITFORD AND LAUNDITCH RURAL DISTRICT (NORFOLK)**; population (1901), 18,437; Dr. Spencer Low. [No. 256, Price 4d.]

*Authority concerned*: Mitford and Launditch Rural District Council.

*Ground of Inquiry*: Repeated outbreaks of diphtheria in the Parish of Guist; request for enquiry by Medical Inspector.

*Chief Facts reported by Inspector*: Some 24 cases of diphtheria reported between December, 1905, and August, 1906. But on investigation a number of unreported cases came to light, which ought to have been discovered at the time of their occurrence. No isolation or disinfection in any useful degree had been practised. Spread of

infection aided by attendance at school of children whilst in an infectious state.

General sanitary administration of the district lax, and revision should be effected in arrangements for nuisance inspection. A hospital for infectious diseases should be provided and a steam disinfecter.

A number of houses in the district were unfit for human habitation. Slop-drainage was commonly in a state of nuisance. Some slaughterhouses were in an unsatisfactory condition.

32. MOSSLEY BOROUGH (LANCS.); population (1901), 13,452; Dr. Fletcher. [No. 265, Price 4d.]

*Authority concerned:* Mossley Town Council.

*Ground of Inquiry:* Inspection in common with a group of eight other neighbouring Lancashire Districts under part-time Medical Officers of Health, none of whom were qualified in Public Health, and five of whom held their appointments without the Board's sanction with a view to repayment by the County Council of moieties of their salaries.

*Chief Facts reported by Inspector:* Water supply from Ashton-under-Lyne, Stalybridge and Dukinfield (District) Joint Waterworks. Solvent action of water on lead mains in town gives rise to some lead poisoning. Water treated with chalk, but apparently insufficiently of late. Some old rubble sewers, but sewers mostly earthenware or iron pipes, or brick culverts. Sewage outfall-works require extension. Excrement disposal is almost entirely by pail or box-closets. Houses generally provided with yard drains. Houses mostly substantially built of local stone, but some old ill-constructed dwellings were seen. Overcrowding in one common lodging-house. Two slaughterhouses well constructed, but two are unsatisfactory. No official register of cowkeepers and purveyors of milk kept. Many of cowsheds unsatisfactory, but some are good. Regulations as to Dairies, Cowsheds and Milkshops are in force. An old farmhouse is rented as a small-pox hospital, but there is no ordinary isolation hospital—the Medical Officer of Health does not deem removal of scarlet fever to be necessary. The Medical Officer of Health receives a salary of £50 a year. The Inspector of Nuisances is not certificated. He also holds office as Surveyor and receives a combined salary of £200 a year, none of which is repaid by the County Council.

33. NEW QUAY URBAN DISTRICT (CARDIGANSHIRE); population (1901), 1,234; Dr. Fletcher. [No. 283, Price 5d.] (Previously inspected and reported on by Dr. Fletcher in 1892.)

*Authority concerned:* New Quay Urban District Council.

*Ground of Inquiry:* The district forms part of the Aberayron Registration District, together with Aberayron Urban and Rural Districts. Inspection of New Quay

and Aberayron Urban Districts was ordered in conjunction with inspection of Aberayron Rural District.

*Chief Facts reported by Inspector:* Water supplies unsatisfactory for most part. Much waste of money on abortive schemes for a public service and on tinkering with existing supplies. Sewerage largely unsatisfactory; improvement has been made by laying certain pipe-sewers, but the outfalls over the rocks and across the sand in the bay are not commendable. Many dwellings are provided with drains, but these are often of defective character. Privy-cesspits have been practically abolished since my former visit, and pail-closets substituted. Houses mostly good substantial buildings, but some old cottages still remain; considerable improvement in this respect. It is said that there are no slaughterhouses in the district. None of the voluntary Acts adopted. Byelaws in force as to new streets and buildings, nuisances, common lodging houses (none in the district), slaughterhouses (none in the district), and pleasure boats and vessels. No regulations as to dairies, cowsheds and milkshops (none in the district). No isolation hospital. Salary of Medical Officer of Health in 1892, £5 per annum! raised after my former visit to £20, at which it now stands. Salary of Inspector of Nuisances only £8 per annum.

**34. PANTEG URBAN DISTRICT (MONMOUTHSHIRE);** population (1901), 7,484; Dr. Johnstone. [No. 276, Price 8d.]

*Authority concerned:* Panteg Urban District Council.

*Ground of Inquiry:* Outbreak of enteric fever in 1906.

*Chief Facts reported by Inspector:* During the period 8th July–10th November, 1906, 56 cases of enteric fever were notified from 40 houses. No marked explosion of cases. Not sufficient evidence to show the cause of the outbreak, but possibly due to occasional access of specifically contaminated water from the gas works spring to the mains and service reservoir supplying the district.

No isolation hospital provided, and no disinfector.

Part of the sewage discharged into the river untreated. Water supply intermittent in dry weather, and hence insufficient flushing of waterclosets in summer.

**35. PONTYPOOL URBAN DISTRICT (MONMOUTHSHIRE);** population (1901), 6,126; Dr. Johnstone. [No. 276, Price 8d.]

*Authority concerned:* Pontypool Urban District Council.

*Ground of Inquiry:* Outbreak of enteric fever in 1906.

*Chief Facts reported by Inspector:* 165 cases of enteric fever in 111 houses notified between 28th April and 22nd December, 1906. Explosive outbreak in August and September; 84 cases in 66 houses notified in the four weeks ended 15th September. 88 of the houses invaded during the whole period found to be within an area supplied with drinking water from the gas works spring. This area contained about half the houses in Pontypool Urban District. Gas works spring found to be contaminated on 31st August, and shut off as a water supply.

Sudden drop in number of houses invaded after 15th September. In absence of other known causes, the outbreak is considered to have been chiefly due to specifically contaminated water from the gas works spring. Personal contact also concerned in the spread of the disease.

No isolation hospital provided ; no disinfector.

Crowding of houses upon area, and overcrowding of persons. Sewers in parts of the town antiquated and dilapidated.

Sewage enters the river untreated. Water supply intermittent in dry weather. Insufficient flushing of waterclosets owing to intermittent water supply.

36. SHEERNESS URBAN DISTRICT (KENT) ; population (1901), without garrisons, 14,293 ; Dr. Reece.

*Authority concerned* : Sheerness Urban District Council.

*Ground of Inquiry* : To ascertain what action had been taken by the Urban District Council to carry out the recommendations made by Dr. Buchanan in his Report on the Sanitary Circumstances and Administration of, and as to the Prevalence of Enteric Fever in the Urban District of Sheerness, 1905.

*Chief Facts reported by Inspector* : Some improvement in the work of the Medical Officer of Health. Appointment of a qualified Inspector of Nuisances, to devote the whole of his time to the duties of his office. Some improvement in connection with the sanitary defects of houses effected by this officer, but no comprehensive action taken by the District Council. Probable diminution of population by reason of large discharges of workmen from the dockyard ; in consequence, less demand for house property, which should enable District Council to deal with insanitary property. Heavy incidence of enteric fever continues, although no pronounced epidemic ; still no records available to enable investigation of its causes.

Serious shortage of water, due to a breakdown of one of the pumping engines ; as a temporary expedient, a new tunnel in course of construction to connect the wells. The water supply will still remain subject to the various and serious disadvantages to which Dr. Buchanan drew attention in his Report of 1905, pp. 9 and 14. Isolation hospital not improved. No improvement in sewage disposal. Code of Bye-laws not revised.

37. SOUTHWICK-ON-WEAR URBAN DISTRICT (DURHAM) ; population (1901), 12,643 ; Dr. Fletcher. [No. 245, Price 5d.]

*Authority concerned* : Southwick-on-Wear Urban District Council.

*Ground of Inquiry* : Continued high mortality from zymotic disease.

*Chief Facts reported by Inspector* : Water supply from the Sunderland and South Shields Water Company. All the sewers are pipe-sewers, which, with one exception, discharge directly into the river Wear by five outfalls. The

largest sewer discharges into a settling tank, whence the effluent passes into the river. House drainage is provided for the majority of the houses by means of yard gullies. Excrement disposal is by "ash-closets," privy-cesspits, or waterclosets; very many old privy-cesspits still in use. House accommodation mostly satisfactory, the majority of fairly modern construction, but many are of very limited capacity. There are some old insanitary dwellings. Of thirteen butchers, only three have slaughter-houses; the remainder slaughter in the shops. Cowsheds fairly satisfactory, but many small unsatisfactory milkshops in the town. Regulations as to dairies, cowsheds and milkshops in force. Ordinary isolation hospital jointly with South Shields Rural District; small-pox hospital jointly with Jarrow Borough, Felling and Hebburn Urban, and South Shields and Sunderland Rural Districts. Zymotic prevalence apparently to some extent due to heavy incidence of childhood's infectious diseases on a population in which the proportion of children is higher than that in the county of Durham, the proportion in Durham being in turn higher than that in England and Wales. Medical Officer of Health does not hold a D.P.H. He is paid £40 per annum.

38. ST. NEOT'S RURAL DISTRICT (HUNTINGDON); population (1901), 7,239; Dr. Sweeting. [No. 253, Price 4d.]

*Authority concerned:* St. Neot's Rural District Council.

*Ground of Inquiry:* As to the need of a whole-time certificated Inspector of Nuisances for this and Eaton Socon Rural District.

*Chief Facts reported by Inspector:* Many cottages in bad state of repair; some overcrowding of persons in them. Bad paving of back yards. Surface wells and open ponds the chief water supply of some places. Many water courses polluted by sewage and house refuse. Need for public scavenging in the larger villages. No proper fever hospital provided; nor disinfectors. Systematic inspection of district neglected by Medical Officer of Health, who is allowed nothing for travelling expenses. Inspector of Nuisances holds a plurality of appointments, which prevents him giving adequate attention to his sanitary duties; a whole-time officer urgently required.

39. TAVISTOCK RURAL DISTRICT (DEVON); population (1901), 16,305; Dr. Sweeting. [No. 240, Price 4d.]

*Authority concerned:* Tavistock Rural District Council.

*Ground of Inquiry:* An outbreak of diphtheria in the families of the officers of H.M. Convict Prison at Princetown, Dartmoor, reported to the Board by the Home Office.

*Chief Facts reported by Inspector:* Most of the diphtheria in prison warders' families; a few cases in families outside the prison. Disease referable not to insanitary conditions, but to evolution from minor sore throat antecedent in the village; largely spread at school. No

hospital provision whatever. Disinfection unsatisfactory. Many damp cottages in the district; some over-crowding in them. Many shallow and dip wells, exposed to serious contamination. Most of the water-closets hand-flushed and filthy. The majority of cowsheds defective in ventilation and paving, and over-crowded. Slaughter-houses dirty, badly paved and drained. Medical Officer of Health an unsatisfactory officer; neglected to report to the Board on the diphtheria outbreak for ten months, and generally negligent and incompetent. Properly certificated Inspector of Nuisances required.

40.\*TROWBRIDGE URBAN DISTRICT (WILTSHIRE); population (1901), 11,526; Dr. Reece. [No. 251, Price 4d.]

*Authority concerned:* Trowbridge Urban District Council.

*Ground of Inquiry:* The Board were not satisfied with the explanation given relative to the new arrangements proposed for the discharge of the duties of Inspector of Nuisances.

*Chief Facts reported by Inspector:* Population mainly industrial. Town a centre of woollen weaving industry. Urban District adequately supplied with good water, and has a comprehensive sewerage system free from privies and midden-privies. Special need for attention to the condition of much old house property, and for effecting improvement in the character of the house drains. Ample work to occupy whole-time services of an active and competent inspector of nuisances, qualified in food inspection. Special need for supervision of the trade in food for man. 500 pigs killed weekly at one slaughter-house, and town is a distributing centre for milk.

In February, 1904, the Board, after inquiry by Mr. Huddart, the Board's Assistant Inspector, wrote asking the Urban District Council to separate the offices of Inspector of Nuisances and Surveyor, at that time held by Mr. Lailey, and to appoint a fully qualified person to the office of Inspector of Nuisances. In June, 1904, the Urban District Council appointed Mr. Strickland as Inspector of Nuisances at a salary of £80 per annum. The appointment was sanctioned by the Board. Mr. Strickland was re-appointed with the Board's concurrence in 1905, but when this latter period of office lapsed he was not again re-appointed by the Council. The explanation given by the Council for not re-appointing Mr. Strickland is that with the completion of the northern portion of the sewage works the several offices of Highway Surveyor, Building Surveyor, Sanitary Engineer, Inspector of Nuisances, and of Factories and Workshops, and Inspector under the Petroleum Acts, and Assistant Surveyor, hitherto distributed among three persons, did not now collectively afford employment for more than two, and that accordingly the District Council proposed, preferring retention of those oldest officers, to appoint Mr. Allen, the Assistant Surveyor, Inspector of

Nuisances as well as Assistant Surveyor at a salary of £100 per annum (£80 as Inspector of Nuisances and £20 as Assistant Surveyor); to secure to Mr. Lailey the posts of Highway Surveyor, Building Surveyor, and Sanitary Engineer, at a salary of £200 per annum; Mr. Strickland to be dispossessed altogether from office.

There would be no diminution of expense to the district should the Board determine to refuse sanction to the appointment of Inspector of Nuisances being held conjointly with that of Assistant Surveyor, as in such case there would be no repayment from the county funds of a moiety of the salary of the Inspector of Nuisances, and the services of a third officer would be lost to the district. The best sanitary interests of a district are not served when an Inspector of Nuisances is subordinated in his work to a Surveyor. Mr. Lailey has been appointed Resident Engineer to the extensive new sewage disposal works, and work in the Surveyor's Department seems to have increased. No systematic work of nuisance inspection done before Mr. Strickland's appointment; excellent work by this officer. The efficiency of the Sanitary Department would suffer unless the whole-time services of such an officer were retained. Mr. Strickland had been dispossessed of office at the date of inspection.

It is unfortunately necessary to consider the circumstances which have led up to the new proposal of the Council from another aspect. The Board received on 12th June, 1906, a letter from Mr. Strickland, with reference to his not being re-appointed as Inspector of Nuisances, in which he states:—"In the performance of my duties I have unfortunately come into conflict with the private interests of several members of the Council. This was unavoidable considering that there are on the Sanitary Committee three large house agents and two butchers . . . I have been interfered with by individual members of the Council when carrying out the details of my duties."

In dealing with old house property Mr. Strickland was brought into relation with house agents, and he has an entry in one of his reports calling attention to interference, by certain of the Council, with him in the execution of his duties, and asking for the support of his Authority. In the matter of food inspection he has seized or caused to be destroyed, with the consent of the owners, unsound food.

An honest opinion may no doubt be held that the sanitary needs of Trowbridge can be met, and diminution of expenditure in administration effected by the appointment of the Assistant Surveyor as Inspector of Nuisances also, but the Board's Inspector is forced to the unsatisfactory conclusion that this has not been the sole factor which has been instrumental in deciding that Mr. Strickland should not be appointed for another period of office.

41. WALMER URBAN DISTRICT (KENT); population (1901); 5,614; Dr. Darra Mair.

*Authority concerned:* Walmer Urban District Council.

*Ground of Inquiry:* Discharge of duties of Medical Officer of Health.

*Chief Facts reported by Inspector:* Annual Reports of Medical Officer of Health inadequate year after year; performance of duties inefficient, limited mainly to dealing with infectious cases; no inspection of houses. Inspector of Nuisances also negligent of house inspection. Absence of flushing arrangements for closets. Isolation hospital inadequate and out of repair. Water supply of Walmer and Deal from same source in the chalk; buildings near site drained to cesspools. Sewers discharge into sea.

- 42.\*WHICKHAM URBAN DISTRICT (DURHAM); population (1901), 12,852; Dr. Darra Mair. [No. 262, Price 1s.]

*Authority concerned:* Whickham Urban District Council.

*Ground of Inquiry:* Representation by Durham County Council that Public Health Act had not been properly put in force by Whickham Urban District Council, in respect of certain back-to-back houses at Marley Hill.

*Chief Facts reported by Inspector:* Census returns show that the proportion of persons in this district who were "overcrowded" was very high—38 per cent. The only districts in England and Wales which have similar records as to "overcrowding" are situated either in Durham or Northumberland. Excessive "overcrowding" an exceptional feature of these two counties. Colliery districts, not situated in these two counties are not overcrowded. Probable reason for these two counties being exceptional is existence in them of the "rent-free house" system. Result is scarcity of houses, and disposition of occupants to put up with inferior houses. Marley Hill back-to-back houses, though possessed of miserable accommodation are occupied by respectable, well-to-do tenants, who express themselves satisfied with their dwellings. Vital statistics for 10 years show that, if Marley Hill be divided into (1) houses which are back-to-back and (2) houses which are not back-to-back, the death-rate from all causes in the former was 16 per 1000, and in the latter 12·6; and that rate of infantile mortality was 221 per 1000 births in the former, and 147 in the latter. Action of Durham County Council in pressing for these back-to-back houses to be made through houses probably right. Whickham Urban District Council should take action to improve housing throughout the district under the Housing of the Working Classes Act.

- 43.\*WIGAN COUNTY BOROUGH, including Township of Pemberton, incorporated November 1904; population (estimated to July 1st, 1905), 88,306; Dr. Copeman. [No. 246, Price 6d.]

*Authority concerned:* Wigan Town Council.

*Grounds of Inquiry:* Continued high infantile mortality-rate, and endemic prevalence of enteric fever and diarrhoea.

*Chief Facts reported by Inspector:* For many years past both diseases endemic in Wigan, owing in large measure to general insanitary conditions, of which long-standing pollution of the soil and subsoil is probably of chief importance. Subsidence in many parts of the town due to coal-mining operations beneath, have frequently caused breakages of sewers and watermains. Huge privy-middens formerly almost universal in Wigan, have been in large measure replaced by pail privies and ash tubs, but scavenging arrangements still inefficient. In former official reports, water supply described as liable to contamination; within recent years, however, constant and satisfactory supply has been provided. Houses inhabited for most part by operative and artisan classes, in many instances exhibit insanitary conditions, especially in the various courts and yards. The town council have found difficulty in dealing satisfactorily with these areas, owing especially to the property being in the hands of numerous small owners.

High infantile mortality rate in large measure dependent on annual prevalence of epidemic enteritis; contributing factors include want of personal cleanliness and improper feeding of infants together with poor quality and undesirable methods of storage of milk. No considerable employment of female labour.

44. WINDSOR RURAL DISTRICT (BERKSHIRE); population (1901), 14,274; Dr. Manby. [No. 241, Price 4d.]

*Authority concerned:* Windsor Rural District Council.

*Ground of Inquiry:* Complaint to the Board that Ascot, Sunninghill, and Sunningdale Villages were much in need of drainage systems.

*Chief Facts reported by Inspector:* Much overcrowding of houses upon area in parts of Ascot, Sunninghill, and Sunningdale Villages. Hence arise difficulties in disposing of faecal filth and slop water. Many gardens found to be "sick" and uncultivated because of too much slop water deposited on them. In other places many cesspools exist in confined situations, and nuisance is alleged from the frequency of their emptying. Tenants also complain of the excessive cost to which this frequent emptying of cesspools exposes them. Improved water supply required for houses in South Ascot, in Sunninghill, and in Sunningdale. More attention required by the sanitary officers to dairies, cowsheds, and milkshops. More systematic sanitary inspection throughout the district is called for. No provision for isolation of infectious cases other than small-pox. Only hospital accommodation for small-pox cases is a small corrugated iron hospital of eight beds. No steam disinfectors available. Rural District Council urged to attend to these matters, and to embark upon a scheme of drainage for at least parts of Ascot, Sunninghill and Sunningdale.

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## APPENDIX A., No. 3.

LOCAL HOUSING CONDITIONS and ADMINISTRATION illustrated  
by EXTRACTS from REPORTS made by MEDICAL INSPECTORS  
in 1906-7.

## WHICKHAM URBAN DISTRICT

(Extracted from a Report by DR. DAERA MAIR).

Whickham Urban District is situated in the north-western corner of the county of Durham on the right bank of the Tyne, just to the west of Gateshead, and opposite Newcastle and Elswick. It is divided from Gateshead by the River Team, though here a portion of the district known as Dunston practically merges into Gateshead. Its western boundary is formed by the River Derwent.

The area of the district is 5,914 acres, or somewhat over nine square miles. The population at the time of the 1901 census was 12,852, having increased from 9,343 at the previous census; but it is estimated to be, at the present time, over 16,000.

This population is not uniformly distributed over the district, but is located in four "towns" or "villages." These places occupy but a small portion of the area of the district, the bulk of which is of an agricultural description.

The "towns" are known as (1) Whickham, which is about the centre of the district; (2) Swalwell, which is in its north-western corner near the confluence of the Derwent with the Tyne; (3) Dunston, which is in its north-eastern corner adjoining Gateshead; and (4) Marley Hill, which is better described as consisting of scattered groups or rows of houses situated in the southern portion of the district.

The industry of the district is almost entirely connected with coal. There are collieries in many parts of the district and in its neighbourhood. There are also flour and paper mills, brick works, and iron works in the district, while the Elswick works on the other side of the Tyne also gives employment to some men living in the Whickham district.

## HOUSING.

At the census of 1901, the number of inhabited houses in the Whickham Urban District was 2,524; and they were occupied by 2,567 families. That is to say, the latter was the number of "tenements" or separate occupations in the district

\* For further particulars relating to the reports from which the following extracts are taken, refer to the Abstract of Medical Inspections, pp. 7 to 30.

according to the census returns. Of these 2,567 tenements, 2,243, or nearly 89 per cent., were "small" tenements, that is to say, tenements of fewer than five rooms each. The corresponding percentages for the county of Durham, and for the urban and the rural districts of the county, were 77·4, 76·5, and 79·8 respectively.

These proportions of small tenements of fewer than five rooms are all very high, as compared with those of the country generally. Thus, at the last census, of all the counties in England and Wales, Northumberland was the only county, except Durham, in which the proportion of tenements of fewer than five rooms to total tenements exceeded 70 per cent.; with the exception of London and the West Riding of Yorkshire, the proportion in question in all the other counties was below 50 per cent. If comparison be made with all county boroughs, and towns (exclusive of London boroughs) with populations exceeding 50,000, of which there were 84 at the last census, it will be found that in only nine of these did the proportion of tenements of fewer than five rooms to total tenements exceed 70 per cent.

These figures show therefore that in these two northern counties—Durham and Northumberland—there is an exceptionally large proportion of small tenements, whether comparison be made with the country as a whole, with other counties, or even with the large towns.

This is noteworthy in itself; but there are other particulars regarding tenements and the number of their occupants in the census returns which are very striking in respect of these two counties, and which it will be useful to consider. Thus, taking first the Whickham Urban District, the returns show that of the 2,243 tenements of fewer than five rooms, 586 were four-roomed tenements, 660 were three-roomed, 886 were two-roomed, and 112 were only one-roomed tenements. They also show the number of occupants of these various classes of tenements. The complete figures are given in Table I. :—

TABLE I.—Total tenements, and tenements of fewer than five rooms, distinguishing those occupied by various numbers of persons, in the Whickham Urban District (Census 1901).

Whickham Urban District.	Rooms in Tenement.	Tenements of fewer than 5 rooms.	Persons per Tenement.											
			1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12 or more.
Total Tenements, 2,567.	1	112	26	24	28	14	9	8	1	2	—	—	—	—
Tenements of fewer than five rooms, 2,243.	2	886	20	130	181	157	111	113	83	56	15	11	7	2
	3	660	10	58	102	117	117	77	81	51	30	6	6	5
	4	586	3	60	87	109	68	86	65	48	27	21	3	8

NOTE.—The table is to be read as follows :—Of the 2,567 tenements, 2,243 were tenements of fewer than five rooms; and of these 112 were tenements of one room, 886 of two rooms &c.; and of the 112 tenements of one room, 26 were occupied by one person each, 24 by two persons each, &c.; and so on as regards the other classes of tenements.

This table serves a very important purpose, for it is possible by means of it to estimate to what extent dwellings or tenements in a given district are crowded or otherwise with occupants, and in this way it throws a significant light on the matter of housing accommodation.

#### OVERCROWDING IN WHICKHAM URBAN DISTRICT.

When the Census Commissioners came to consider the "tenement statistics" of the country, they dealt with them mainly with reference to "overcrowding," and they fixed upon a standard to enable them to estimate the proportion of persons who were overcrowded in their dwellings. This standard assumes that tenements which are occupied by *more* than two persons per room ("room" being taken to include every bedroom, sitting-room, or other room) may be considered to be overcrowded—a standard which there can be no doubt is a very reasonable one on the whole.

Putting aside for the moment, however, whether this standard strictly applies to Durham, it can be calculated from Table I. that in the Whickham Urban District 62 of the one-roomed tenements, 398 of the two-roomed, 179 of the three-roomed, and 59 of the four-roomed tenements were inhabited by more than two persons per room. Stating it in another way, it will be found that 698 families, or 4,889 persons, were housed, so that they were living more than two persons per room. Those interested can calculate from the table how many of the tenements were occupied by more than three, four, five, or even six persons per room.

The proportion of persons, however, living more than two per room, and classed by the Census Commissioners as "overcrowded," which is disclosed by these figures, is very large indeed. The number of "overcrowded" families is 27 per cent. of the total families in the district; and the number of "overcrowded" persons (4,889) is as high as 38 per cent. of the total population of the district.

In England and Wales as a whole, the proportion of "overcrowded" persons to the total population was but 8·20 per cent., while in the aggregate of county boroughs it was 8·30, in that of urban districts 6·56, and in that of rural districts 5·84 per cent.

#### COMPARISON WITH OTHER DISTRICTS.

But in order to appreciate more clearly how high the proportion of "overcrowded" persons in the Whickham Urban District is, reference should be made to Appendix A of the General Report on the 1901 Census, Table 42. This table gives, amongst other information, the proportion per cent. of "overcrowded" persons to the total population in the case of every county, county borough, metropolitan borough, and town with a population of more than 50,000; and examination of its figures will show that in not one of these localities was the proportion of persons living more than two in a room so high as it was in the Whickham Urban District.

**OVERCROWDING IN DURHAM AND NORTHUMBERLAND AS  
COMPARED WITH ELSEWHERE.**

Exclusive of London there were at the time of the census 145 counties, county boroughs, and large towns. In 120 of these (83 per cent.) the "overcrowded" population did not exceed 10 per cent.; in 18 (12 per cent.) it exceeded 10, but did not exceed 25 per cent.; while in seven only (5 per cent.) it exceeded this proportion.

These seven exceptional localities were as follows:—

Counties.	County Boroughs.	Large Towns.	Overcrowded Population per cent.
Durham ... ..	—	—	28·48
	Sunderland ... ..	—	30·10
	Newcastle-on-Tyne ... ..	—	30·47
	—	Tynemouth ... ..	30·71
Northumberland ... ..	—	—	32·09
	South Shields ... ..	—	32·42
	Gateshead ... ..	—	34·54

The fact that these high proportions are restricted absolutely to Northumberland and Durham, and to certain places within their borders, is very remarkable; and it suggests, at first sight, some connection with the coal-mining industry, as, indeed, the Census Commissioners were inclined to conclude in their General Report. Further consideration, however, shows very clearly that this is not the case.

**COMPARISON WITH OTHER COLLIERY DISTRICTS.**

I have prepared a table (not reproduced) on the lines of Table 42 in the Census General Report, already alluded to, and I have placed along with the statistics relating to the county of Durham and the Whickham Urban District those relating to Lancashire, Yorkshire, and Northumberland; those relating to certain typical colliery districts in Yorkshire, Lancashire, Derbyshire, Worcestershire, and South Wales; and also those relating to certain industrial centres not connected with coal.

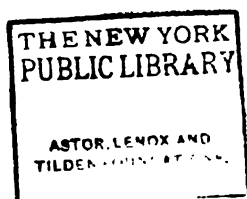
The essential facts of this table are shown in diagrammatic form on the annexed plan. It will be seen that in not one of the selected colliery districts outside Northumberland and Durham was the proportion of those living more than two in a room ("overcrowded") anything approaching the high proportion characteristic of those two counties; that while in Dudley, where the proportion was highest, it was 17·48 per cent., in Heanor and Rhondda, where it was lowest, it was only 3·58 and 4·98 per cent. respectively. It will also be seen that, outside Northumberland and Durham, there is no material difference in this respect between coal districts and districts not connected with coal.

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lead them to bear with, such lower standard.



difference in this respect between coal districts and  
not connected with coal.

. . . . .

It would be instructive to pursue this matter still further in order to compare the relative preponderance of the one, two, three, and four-roomed tenements in the different districts, and to ascertain the ratio of "overcrowded" persons in each class of these tenements to the total population and to the total "overcrowded" population in each of the districts; indeed, these tenement statistics would well repay much study. But this would take up much time and is, perhaps, scarcely needful for the purposes of this report.

It will suffice to point out that in the Whickham Urban District, as in the counties of Northumberland and Durham, it is the two-roomed tenement which preponderates, although in Ashington, which is one of the newest colliery districts in Northumberland, the three-roomed tenement largely prevails; while in the colliery districts belonging to Lancashire and Yorkshire it is the four-roomed tenement which exists in the highest proportion; and in the colliery districts of South Wales and of the Midlands, and in an industrial centre like Preston, tenements of five rooms and more are relatively the most numerous, as they are in England and Wales generally.

The diagram shows these variations at a glance, and makes apparent the very striking difference between Northumberland and Durham and places within their borders, and places outside their borders, both in respect of the preponderating classes of tenements, and in respect of the overcrowding.

#### EXCESSIVE OVERCROWDING AN EXCEPTIONAL FEATURE OF NORTHUMBERLAND AND DURHAM.

The broad facts which appear from the foregoing are that in the Whickham Urban District the proportion of small tenements, and particularly those of two rooms, is extremely high as compared with colliery districts and industrial centres in other parts of provincial England and Wales; that the proportion of its inhabitants living more than two in a room, and classed by the Census Commissioners as "overcrowded," is also very excessive when compared in similar fashion; and that these two exceptional conditions are a feature of Northumberland and Durham generally, and of these two counties only. Stated in another way, it seems to be the fact that the people of the Whickham Urban District, and those of Northumberland and Durham generally, not only live in very small houses, that is to say, in houses with but few rooms, and that they live much crowded together in these small houses, but also that there is no other district in provincial England and Wales to compare with or even to approach them in these respects. The deduction seems inevitable that for such very exceptional conditions to exist in Northumberland and Durham, either the people there must have a lower standard of comfort and decency than those elsewhere in the country, or social and economic forces must be at work there, the effects of which accustom the people to, or directly and indirectly lead them to bear with, such lower standard.

## THE "RENT-FREE HOUSE" SYSTEM IN NORTHUMBERLAND AND DURHAM.

Now it is the case that there is in existence in Northumberland and Durham, or at all events in so far as the coal industry is concerned, an arrangement in regard to house accommodation which, so far as I know, does not exist in any other industrial locality outside these two counties. By this arrangement, most of the heads of families employed in and about the coal mines are provided with house accommodation by their employers, rent-free, or, in the absence of such house accommodation, with a rent-allowance in the shape of an addition to their wages.

Although this arrangement became the subject of a definite agreement between masters and men so lately as some six years ago, this was only for the purpose of settling the practice, as it were, for it is in fact based on a custom which dates back indefinitely. It is said, indeed, that it is a relic of the feudal system. The northern coalfield in these two counties is at any rate the most ancient in this country. It is believed that the first licence to dig for coal there was granted in the reign of King John, and in those days it was the custom of the lords of the soil to provide dwellings for their dependents. So, it is said, this custom began, has been handed on through the centuries, and remains to this day, a singular feature of this coalfield.\*

On the other hand, all other coal centres are relatively of very modern development, within, say, the last two centuries, dating from a time when this feudal custom had largely disappeared: and this is believed to be the principal explanation of the fact that in all coalfields, except that of Northumberland and Durham, the normal house-rent system prevails. That is to say, in all these other coal centres, there is the usual relationship between landlord and tenant, and house rent is payable by the miner, even though his employer be, as is often the case, the owner of the house, too. It is a separate transaction altogether.

The outcome of this exceptional feature of the northern coalfield is somewhat difficult of comprehension, and is, I understand, the subject of a great deal of difference of opinion amongst those intimately acquainted with the coal industry. I gather, however, that until the early seventies of the last century, the system worked smoothly enough, for up to that time the supply of houses belonging to the coal owners was about sufficient for the number of families employed; but at this period the great boom which occurred in the coal

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\* "Smart money" is said to be another relic of the feudal system. This is the term applied to the payment allowed in this coalfield since ancient times by the employer to his workman when injured in the performance of his work; and although this payment has been superseded now by that required to be made under the Workmen's Compensation Act, "smart money" nevertheless remains in the shape of a payment of 5s. per week by the employer during the first fortnight following the date of the injury, for during this period nothing is payable under the Compensation Act referred to,

industry as a result, it is said, of the Franco-German war, led to a great and rapid increase of the number of persons employed. This soon caused the demand for house accommodation to out-grow the supply, and many came to be housed in dwellings, not the property of the employers, for which, of course, rent had to be paid.

An allowance for rent, in place of a rent-free house, was the natural outcome of this development. The amount added to the wages as rent-allowance in these cases varied in different districts, but it may be assumed that, although perhaps diverse considerations took effect, it was fixed in each place at a sum which at that time, more than 30 years ago, represented approximately the market value of the houses which had to be rented. The sum then so fixed in the different districts has, speaking generally, remained the same to the present day, and so it comes about that now the "rent-allowance" is by no means uniform throughout the coalfield. It ranges from 1s. 6d. to 3s. 3d. per week, but in 80 per cent. of the cases the amount paid is 2s. to 2s. 6d. a week, the average for the county of Durham being 2s. 4½d. It remains to be added that a rent-free house or rent-allowance carries with it in either case a supply of coal for use as fuel in the house, free of cost, but for a small sum (6d. a fortnight) the cost of cartage of the coal.

In order to clearly appreciate the position in regard to this matter, it must be remembered that, as I have already said, only heads of families are entitled to "free" houses, or to the allowance for rent in lieu thereof.\* A miner's grown-up son, for instance, if unmarried, may be paid the same rate of wage as the father, but he does not receive any additional sum to represent either the value of the house supplied to his father or the rent allowance which may be given to the latter in lieu thereof.

It is obvious that, if a system such as is above described is to work smoothly, either the supply of houses belonging to colliery owners—in other words the supply of "rent-free" houses—must always be equal to the demand; or, the "rent-allowance" given in lieu of a free house must be sufficient to pay the rent of the house which has to be taken; but, as may be expected, neither are there sufficient "free" houses, nor in most cases does the "rent-allowance" meet the charge made for a rented house. Not only has the market value of the rented house risen in many cases by the ordinary effects of the law of supply and demand, and not only have rates and taxes increased, but also while it may have been possible 30 years or more ago to build houses remuneratively to let at, say, 2s. per week, it is probably not possible to do so now, owing partly to the rise of wages in the building trade, and partly to the necessity of providing better houses. In the Whickham Urban District, for instance, the rents of houses

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\* This privilege is, moreover, limited to certain "classes" of workmen, but for practical purposes it applies to 90 per cent. or more of the heads of families.

built within recent years range from 4s. 6d. to 6s. per week, and these rents, I understand, are not exceptional for the county of Durham.

It follows, therefore, that the miner who is in receipt of "rent-allowance" is worse off as regards his net income than his fellow-worker who has a "free" house to the extent of the difference between the "rent-allowance" and the rent he has to pay, and that this difference may amount to as much as 3s. 6d. or even more per week. And this is not all. For if the miner with a "rent-allowance" receives no wage owing to absence from work from any cause such as sickness,\* holiday, trade dispute or what not, he, likewise, receives no "rent-allowance," although, of course, the rent continues to be payable; whereas the miner with the "free" house, though he also receives no wage under similar circumstances, remains in possession of his house "rent free."

Furthermore, in the event of a miner being incapacitated from work by injury received in the course of his work, the amount of compensation to which he is entitled under the Workmen's Compensation Act is based on his wage and, if he is the occupier of a "free" house, on the calculated rental value of that house, as well as on the calculated value of the coals with which he is supplied free of cost. By agreement between the masters and the men the "value" of the "free" house and coals has been fixed for this purpose at 5s. per week, 3s. for the house and 2s. for the coal, and this "value" applies uniformly to the whole of Northumberland and Durham. On the other hand, in the case of a miner with "rent-allowance," the basis of his compensation in the event of injury is his wage, his free coal, and his "rent-allowance," which, as I have before stated, is, generally, less than 2s. 6d., and therefore appreciably less than the "value" of the "free" house as calculated above.

It is thus apparent that there are several anomalies in the system of "free-houses" for miners as it exists at present; and, as may be expected, it is not now altogether satisfactory either to the owners or to the men. It is, perhaps, only natural that the men desire the owners to increase the number of "free" houses by purchasing dwellings which do not already belong to them and, if necessary, by erecting additional houses; while, on the other hand, it is not surprising that the owners evince an increasing disinclination to act in this way, and desire that the whole system of "free" houses should be brought to an end and replaced by the normal system which prevails in every other industrial centre. The men, however, resist this proposal, for they not only look upon the "free" house as an "inalienable right" which has descended to them through centuries of practice, but they realise that, whatever the disadvantages of the system may be from their point of view, the advantages of having a rent-free house when no wages are being earned, which may sometimes be for very

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\* In some districts "rent-allowance" is paid in some cases during sickness.

long periods such as in times of sickness and in times of strike, are too solid to be lightly given up, and that these advantages could not probably be obtained under any other system.

#### EFFECTS OF THE "FREE HOUSE" SYSTEM ON HOUSING.

I have discussed this matter somewhat fully because it is necessary to state the facts in order to comprehend how this system of free houses bears on the question which it is the purpose of this report to deal with—the housing accommodation in one of the colliery districts of Durham. I think the following considerations will show that its bearing is a very real one, and that it goes a long way towards explaining how it is that, as the census returns indubitably show, the people of the northern coalfield are living in such exceptionally bad conditions of housing and of crowding together in their houses.

#### INTERFERENCE WITH BUILDING BY PRIVATE ENTERPRISE.

In the first place, the very fact that proprietors of collieries are known to be responsible for the provision of houses for their workmen must interfere with the building of houses by private enterprise. Anybody who builds houses, does so in the expectation that he will obtain a return for his capital so expended, and it seems obvious that he would not be attracted to a place in which he knew that other persons had built houses or might build houses, let or to be let for no rent at all.

Of course there would always be people even in such places who would not be entitled to "rent-free" houses—tradespeople, for instance,—and who would require house accommodation; so that there would always be need for some building by private enterprise. And this is the reason, no doubt, why numbers of the houses do not belong to colliery proprietors. Furthermore, it is conceivable that individuals, relying on the expectation that colliery owners would not build a sufficient number of houses, or that they might buy houses already built, might be tempted to embark their capital on building some houses in the hope that a satisfactory return would ultimately be forthcoming. It needs but little reflection, however, to see that this is a very different thing to the amount of building by private enterprise which might be looked for in a district, without the "free" house system, in which, say, a new pit was being sunk, or in which a considerable extension of an existing pit was contemplated, or in which from any other cause an increased demand for dwellings was anticipated.

It seems also obvious that, even when houses are built by private enterprise, they would approximate fairly closely in character to the "rent-free" houses erected by colliery owners—there would be no incentive, at any rate, to compete with them and to provide better houses at the same rent, such as might be expected in places where there was no "free" house system.

#### TENDENCY OF COLLIERY OWNERS TO BUILD AS FEW HOUSES AS POSSIBLE.

In the second place, it seems but natural that colliery owners should seek not to build more houses than are absolutely necessary, seeing that they receive no rent for them; and also not to lay out more capital than is absolutely necessary on the houses which they do build. As a consequence, houses have been built with as few rooms as possible. This phase of the question is more acute than it was even 30 or 40 years ago, since it costs more to build a house now.

#### MINERS ANXIOUS TO BE SATISFIED WITH "FREE" HOUSE IF POSSIBLE.

It will seem to most people that these two results of the free-house system must be inevitable, and that they alone must have an enormous influence on the housing accommodation generally. On the one hand, there is the miner anxious to have a free house if there is one available, or, if there is not, anxious for a house the rent of which is as nearly equal to his rent allowance as may be; on the other hand, there is the owner anxious to meet his obligation to provide house accommodation for his men with as little capital sunk in the process as is possible, and the private builder unlikely, under the circumstances, to risk his capital unless he sees there *must* be a demand for his houses or unless he is prepared to get but small return for his capital.

The two factors tend, unfortunately, in the same direction. One a natural desire to build as few and as small houses as possible, the other an equally natural tendency to be satisfied with such accommodation as these houses can be made to yield. With the operation of two such factors extending over a great length of time, it becomes no matter for surprise that the standard of comfort and decency among the people generally is, as the census returns indicate, lower than that prevailing among the working class generally, and also lower than that prevailing among those engaged in similar occupation in colliery districts elsewhere.

#### EVIDENCE OF MARLEY HILL COTTAGES.

No better example of the effect of the factors above referred to could, perhaps, be found than the case of the Marley Hill houses, which brought about this inquiry. These dwellings, which are all "rent-free," are rows of back-to-back houses, without, of course, means of through ventilation; and one of the points strongly insisted upon by the Whickham Urban District Council as a reason why it is unnecessary to terminate their back-to-back nature, is that most of them have been occupied by the same families for many years, that these people are of a superior class, unlike those generally associated with back-to-back dwellings, and also that the majority of these people are quite contented with their accommodation.

These statements are made by those who have known and have had dealings with these people intimately for years, and I have no reason at all to question their accuracy. Yet each of these families is living in a house which is not only back-to-back, but which has very poor accommodation, consisting, with very few exceptions, of two rooms only, one above the other.

The lower "living" room is entered directly from the air outside, and is used as the kitchen, the living room, the washing room, and generally also as a bedroom; while the upper room is a low room with an attic ceiling, without fireplace, with a small window level with the floor, and approached from the room below by a step-ladder, the top of which emerges through a square hole in the floor which is usually unprotected by a door or otherwise.

Most of these houses of two rooms are occupied by a large number of persons, seldom by fewer than four persons each, and sometimes by as many as eight, nine, ten, and even twelve persons each. The occupants, moreover, are often all adults, and frequently the upper bedroom is used, in common, by two or more adult brothers and sisters, one of whom, in a case I came across, was married and had an infant. Occasionally the upper room is divided into two by a partition, with the result that the two rooms so provided are exceedingly small and the possibilities of ventilation are still further reduced.

The lower room is, as I have said, generally utilised as a bedroom. An article of furniture very common here and, indeed, in most colliery districts in Northumberland and Durham, is a wardrobe or cheffonier, often highly ornamented, which at night can be opened out to become a bedstead. The general use of such a piece of furniture is, in itself, eloquent proof of the scantiness of the room accommodation.

This lower room is used, too, for the washing which a miner generally subjects himself to after work, and though this may involve well-nigh complete stripping of his clothing, it is quite a common spectacle to see it being done in the presence of other members of the family, including those of the opposite sex.

The houses themselves were generally very clean and well, though often too fully, furnished, and apart from overcrowding there was a noticeable absence of those squalid conditions inside the dwellings which are frequently observed in back-to-back houses. But the surroundings of these houses were in most cases bad. As is usual with back-to-back houses, it is practically impossible to provide closet accommodation to be convenient for everybody, and what there is, is insufficient. The roads between the rows are not made up, and their surface is mud in wet weather. The slop drainage arrangements, likewise, are not satisfactory, sometimes being merely open channels, and the paving of the space in proximity of the houses is often defective.

These houses were built some 80 years ago. They are occupied by families who, as I have said, are looked upon as

eminently respectable, many of whom have lived in them for generations, and who are contented with their surroundings. Many of the families contain several workers, so that the combined wages earned in some of the households must be very considerable. I venture to say, without much fear of contradiction, that if the normal system of rented houses prevailed here, these very people would not tolerate such accommodation, and that the houses, if they remained in existence at all, would be occupied by a much lower grade of society.

#### SIMILAR EVIDENCE ELSEWHERE IN WHICKHAM URBAN DISTRICT.

Similar evidence came to my notice in other parts of the district. In Swalwell, for instance, I came across several cases of families living in tenements, some of them wretched places, where inquiry showed that very considerable wages were being earned, but that the occupants chose to live there "rent-free" rather than have a rent-allowance and, by paying a little more, live in one of the better houses which have been built in this township in recent years by private enterprise. One case, in particular, was very striking; that of a family who had been obliged to live in a rented-house, but a rent-free house having become available, they had just moved into it, although the former was a fairly commodious modern self-contained house, while the latter was a wretched tenement consisting of two rooms only in a house largely constructed of wood, which comprised several similar tenements. And yet this family were earning good wages; but they had been tempted by the prospect of having a rent-free house to leave a house the rent of which cost them in addition to their rent-allowance perhaps 2s. 6d. per week. Again, it is almost impossible to believe that, under normal circumstances, with no temptation of having a "free" house, these people would have dreamt of putting up with the accommodation provided in the dwelling into which they had just moved.

#### OBLIGATION TO ACCEPT "FREE" HOUSE, IF OFFERED.

This tendency to accept if they possibly can the accommodation of the "free" house is, moreover, technically an obligation to do so, although I did not understand that this was the case in the instances to which I have just referred. There is a clause in the agreement which was made between masters and men six years ago, stipulating that should a miner refuse to accept the "free" house offered him he is not entitled to any "rent-allowance." It is easy to see how such a rule, which may be desirable and even necessary as part of a system of "free" houses, must needs intensify the evils which I have been describing, and may even at times be used to coerce families into living in houses which otherwise they would decline to live in. Moreover, in consequence of the houses being numerically as few as is possible for the reasons which I have already given, it may so happen that there is no house for a miner other than that offered by his employer in the

place where his work lies, and he is thus compelled to live in a house, however unsatisfactory it may be, which, if there had been a free market in houses, as it were, he might not and probably would not have been obliged even to consider as a possible residence for his family.

#### RENT OF BAD HOUSES INFLATED.

And I might mention here that I was impressed in the Whickham Urban District by the way in which rents for privately owned houses were seldom less than 2s. 6d. Although a man may have to pay more, sometimes a good deal more, than his rent-allowance for his house, especially in the case of those recently built, he is seldom called upon to pay less than the whole of his rent-allowance, no matter how old and dilapidated the house rented by him may be, and no matter how unsatisfactory and poor the accommodation. In other words, just as the "free" house system tends to keep down rents in the case of better houses and thus tends to prevent their erection, so it also tends to *keep up*, at any rate to the level of the rent-allowance, the rents of bad houses, even of those which might well be described as hovels.

#### OTHER EFFECTS OF THE "FREE-HOUSE" SYSTEM.

Another serious feature, to my mind, of the system in practice is the outcome of a working rule, which seems natural and proper in itself, that as the "free" houses are limited in number, those families which comprise the greatest number of workers shall have the first claim on these houses. Thus, it comes about that a family consisting, say, of the father and two or three adult sons, workers, would have a prior title to a "free" house to a family consisting of father and, say, five or six small children; that is to say, in the former case, a family which might be earning £6 per week, or more, would have no rent to pay, although in the latter case, a family which could only earn one man's wages, might be obliged to pay, in rent, some sum varying in amount in excess of his rent allowance, if no "free" house was available for him. This is obviously a complete reversal of what would obtain where the "free" house system is not in existence, and seems to be a direct incentive to overcrowding.

Yet another serious outcome of the system, and one, moreover, which has an important bearing on the sanitary administration of a district, is the power it gives to the owners of "free" houses to stifle complaints by their tenants of the insanitary condition of their dwellings. I am told that this is not an infrequent occurrence. It is at least not difficult to see that the owner may turn out or threaten to turn out of their dwellings those whom he knows to have made such complaints, and although, of course, this may be as easy in a district where there is no "free house" system, yet in the former case it may have much more serious results, for it means at least the renting of a house costing, may be, more

than the rent allowance, while, owing to the paucity of houses which results from the system, it may mean the loss of his employment in consequence of inability to get another house at all.

The "free house" system also seems to introduce complications in local government. The owners complain that, owing to the system, the men are in no way concerned with the payment of the local rates, not even indirectly, although, of course, their voting power far outweighs that of the owners, and that as a consequence expenditure falling on the rates is often extravagant. On the other hand, it is said that sanitary officers of the local authorities often find themselves hampered in enforcing necessary improvements in houses owing to the influence of the presence on these bodies of colliery managers and others interested in the owners' point of view. And it is said that, owing to the same influence on benches of magistrates, this difficulty extends to the enforcement of sanitary requirements by Magistrates' Orders under the Public Health Act, 1875, or by closing orders under the Housing of the Working Classes Act, 1890.

Furthermore, the men are as much interested as the owners in preventing the closure by Magistrates' Order of insanitary houses if they are "free" houses; for such closure involves the removal of the occupants to other houses for which rent may have to be paid; or, if there are no other houses, tends to the loss of their employment.

What may be termed a subsidiary difficulty often arises, moreover, in getting sanitary improvements, which have been "ordered," carried out within a reasonable time. Not only is this due to the fact that the majority of houses in a given locality belong to one person, but also because that person almost invariably employs his ordinary workmen for that sort of work, and these men are not always available. This matter was tersely put to me in this way:—"When trade is bad the owners excuse delay on account of expense, and when it is good they say that they have not time," which means that their staff is more remuneratively employed in other work, namely, digging coal.

#### EFFECTS OF THE "FREE HOUSE" SYSTEM SUMMARISED.

Such are the objections to this "free-house" system which I have been able to gather during a brief investigation of the matter, and, doubtless, there are others. So far as the owners are concerned, it is imposing a severe and an unusual tax on their capital. So far as the men are concerned, the system is compelling them, directly and indirectly, and inevitably, as it seems to me, to live—it would hardly be too much to say, to herd—in houses which are too few and too small for their families, in such a way that no place in the provinces outside Northumberland and Durham can be compared with them.

If the one consideration of having a "rent-free" house in times of sickness or strike could be excluded, I question

whether there would be any hesitation on the part of the men in abandoning the system. Indeed, I doubt not that, in the absence of this consideration, the system would be gratefully discarded by the common consent of everybody. The consideration in question is, no doubt, a very heavy one, but my very strong impression is that the advantages underlying it, great though they undeniably are, are being too dearly purchased, and that, in spite of them, the men would be well-advised in their own interests to bring the system to an end.

At present, however, they will not do so. Recently the Northumberland miners have voted "dead against" its abolition. The system is there, and even if it could be abolished to-morrow its effects would last for some time; so that the housing question has to be dealt with in spite of it.

#### DIFFICULTIES OF DEALING WITH THE HOUSING QUESTION ENHANCED BY THE SYSTEM.

There is no doubt, however, that the difficulties of dealing with the housing question in Durham are enhanced by the existence of the system of "free" houses. Both masters and men are, as I have pointed out, interested, from different motives, in hindering the closure of bad houses; and thus both may also be said to be interested in hindering attempts, on anything like an adequate scale, to improve the sanitary condition of dwellings, lest such attempts should lead to closure. Similarly, owing to the paucity of houses which is, as I have endeavoured to show, a direct and inevitable result of the system, there are great and obvious difficulties in the way of dealing with the question of overcrowding in serious fashion. Difficult as it always is to deal with overcrowding in thickly populated localities, it becomes doubly so where action against it involves in most cases removal from a "free" house to one where rent has to be paid, for this brings with it a transaction objectionable to both master and man, the former having to pay "rent-allowance" where he did not have to pay it before, and the latter having to pay rent more or less in excess of his rent-allowance; and the difficulty becomes still greater when, as may frequently be the case, there is no available house, whether rent free or not rent free, for the overcrowded family to occupy, and action, therefore, may involve loss of employment.

Moreover, this very paucity of houses with the consequent overcrowding, acts in itself as a very powerful obstacle to the closure of insanitary houses, as may readily be imagined. Closure means, of course, displacement of the occupiers, and this involves again the difficulty of obtaining accommodation elsewhere in an already overcrowded locality, a difficulty which has frequently proved insuperable.

Again, as a little reflection will show, the "free house" system renders it specially difficult to deal with the housing question by the adoption of that portion (Part III.) of the Housing of the Working Classes Act, 1890, which enables the

local authority themselves to build houses. Colliery proprietors, averse as they already are to public expenditure to which the men, in consequence of the system, do not contribute, even indirectly, by way of rates, naturally use their influence to prevent expenditure in this direction, since it would practically mean the provision of extra house accommodation at their cost, and this, not only without the prospect of any benefit to them, but with the prospect of it actually leading to increased cost to them by way of more rent-allowances, and may be also by the emptying and disuse of some of their own houses and, consequently, the disuse of the capital sunk in those houses.

On the other hand, the men cannot be expected to welcome enthusiastically the provision of such houses, for at the best such provision would mean an increase of the number of houses, to be secured only by paying for them, in rent, more than their rent-allowance, unless, of course, they were to be rented at a figure not in excess of the rent-allowance. But this would, no doubt, mean that such houses would be a considerable burden on the rates, which would in turn naturally involve the still more strenuous opposition of the colliery proprietors.

#### ACTION OF THE DURHAM COUNTY COUNCIL.

The situation, therefore, seems to be something approaching an impasse. Nevertheless, something is being done to remedy matters. The Durham County Council, under the advice of Dr. Eustace Hill, their Medical Officer of Health, have done much under the powers conferred upon them by the Housing of the Working Classes Act, 1890.

Quite recently the County Council have taken an important step which I believe is the first of its kind. They have decided to put in force Part III. of the Housing of the Working Classes Act, 1890, in a part of one of the rural districts (Chester-le-Street) in default of the local authority; and it is their intention to build houses themselves there as rapidly as may be, as the local authority will not do so. This is being done under the Housing Act of 1900 (Sec. 6), which enables the county authority so to act on a resolution of the Parish Council; but this power, again, is limited to rural districts only.

#### ACTION BY DISTRICT COUNCILS.

I am not in a position to say whether action by the District Councils, urban and rural, has been considerable, inconsiderable, or negligible, in this direction, during the same period. The information at my disposal tends to indicate that such action on their part has been, at least, not very considerable, and that the local authorities have been readier to embark on large schemes of sewerage, paving of streets, provision of

public offices, and so forth—which, no doubt were very desirable—than they have been to take either sustained action to improve the existing house accommodation or to attempt housing schemes. Only two authorities in the county of Durham have made any attempt in this direction by adoption of Part III. of the Housing of the Working Classes Act, namely, the Stanley Urban and the Sunderland Rural District Councils.

#### ACTION OF WHICKHAM URBAN DISTRICT COUNCIL.

In regard to the Whickham Urban District, but little evidence was adduced to me to show that much has been done to improve the housing of the population so far as the District Council is concerned. Action has, it is true, been taken by their officers to improve the housing conditions in respect of nuisances, provision of closets and ashpits, and to some extent also in respect of the paving of yards and of private streets, but as regards the dwellings themselves but little seems to have been done by them.

Some 10 years ago the housing question became so acute in Swalwell that the Urban District Council decided, unanimously, to take steps to adopt Part III. of the Housing of the Working Classes Act, 1890, with a view to building houses there, and a committee was formed to advise the Council; but eventually, after a year's consideration of the matter, the proposal to adopt the Act was negatived and the matter dropped. Nevertheless, even this abortive action seems to have done good; at any rate, it is claimed that in consequence of it, private enterprise stepped in and a considerable number of houses have since been built in this township. This supplies a good example of how, in spite of the "free house" system, the private owner will erect houses if he is convinced that there is bound to be a demand for them.

It seems to me that as, in consequence of the exceptional circumstances of this coalfield which I have discussed, the working people are wedded, if one may say so, to housing conditions which are altogether lower than those found elsewhere in this country, and as, from different and quite as natural motives, the employers cannot be expected, situated as they are at present, to mend matters in this respect, it becomes all the more important for the sanitary authorities to do everything in their power to ameliorate these housing conditions, even against the inclination, if need be, of both parties. If sanitary authorities will not or, for electoral reasons, cannot seriously attend to this business, it is difficult to see how, under the circumstances of the case, housing conditions in this part of the country can be expected to do other than remain at a sort of dead-level and compare worse and worse, as years pass, with the remainder of the country.

At the same time most dispassionate observers will, I think, agree that the speediest way to bring about real improvement in the housing conditions in this district, and throughout the northern coalfield, lies in abandoning the "free house" system which so seriously entangles the matter, and that this system is, on a balance of considerations, deeply injurious to the interests and well-being of the people most concerned, viz., the miners.

Table showing the population and deaths in the Whickham Urban District, the back-to-back houses in the Marley Hill Sub-district, and in the remainder of Marley Hill, respectively, in each of the 10 years 1896-1905, and the average annual death-rate per 1,000 living in these three areas, together with the death-rate of England and Wales for each year of the same period.

Year.	England and Wales.	Whickham Urban District.		Marley Hill Sub-District.			
				All back-to-back houses.		Remainder.	
	Death-rates per 1,000 living.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.
1896 ...	17.1	9,870	176	718	17	1,100	15
1897 ...	17.4	10,010	216	718	10	1,100	19
1898 ...	17.5	10,150	216	718	9	1,100	15
1899 ...	18.2	10,300	206	718	11	1,100	10
1900 ...	18.2	10,440	234	718	11	1,100	14
1901 ...	16.9	12,857	320	718	17	1,100	23
1902 ...	16.2	13,752	212	718	9	1,200	12
1903 ...	15.4	13,808	261	718	13	1,200	13
1904 ...	16.2	14,255	258	718	10	1,292	18
1905 ...	15.2	14,717	188	718	8	1,292	7
Totals ...	—	120,159	2,287	7,180	115	11,584	146
Average Annual Death-rates.	16.83	19.03		16.02		12.60	

NOTE.—The population figures are those given by the Medical Officer of Health and the local Registrar, estimated in the case of the urban district, ascertained by special census for Marley Hill. Calculated, by the Registrar-General's method, on the basis of intercensal increase between 1891 and 1901. I estimate the annual average population of the urban district during the above period to have been 12,675, which makes the death-rate to have been 18.04.

The deaths allotted to the urban district are inclusive of 51 residents who died outside district, and exclusive of three non-residents who died in the district; but the Marley Hill deaths have not been corrected in this way.

## CHESTER-LE-STREET RURAL DISTRICT

(Extracted from a Report by DR. W. W. E. FLETCHER).

## HOUSE ACCOMMODATION. OVERCROWDING.

Proper accommodation for the housing of colliers and their families at the numerous "pit-villages" throughout the district is, unquestionably, urgently required; and the methods of securing its provision have latterly been seriously considered by both the Durham County Council and the Chester-le-Street Rural District Council. . . . . The typical "pit-village" exhibits long rows of low brick or stone houses, the rows being divided from one another by roadways, sometimes of considerable width, formed of merely the ground trodden hard. In wet and wintry weather these passage-ways become dirty and muddy, while in dry and windy weather clouds of coal-dust are driven about the houses like blown sand along the sea-shore, and even indoors furniture and other articles become covered with thick layers of dust and objectionable matter mingled therewith. . . . . It has been known to the Board for some time that the County Council have been urging the District Council to take action under the Housing of the Working Classes Act, 1890; and that reports have been made by Dr. Taylor, the Medical Officer of Health, which have clearly demonstrated the urgent need for reform; and, further, that the justice of Dr. Taylor's reports has been confirmed by a Special Committee of the District Council who subsequently visited certain localities in Usworth and Washington with him and satisfied themselves that the circumstances really were as Dr. Taylor had represented.

Having visited all parts of the district, and having inspected very many houses of the different types mentioned, I feel myself quite justified in fully corroborating Dr. Taylor's representations. Not only are large numbers of houses to be condemned as unsuitable for families, especially for those comprising adolescents of both sexes, on account of insufficient accommodation and consequent overcrowding, but many of them are in more or less obvious disrepair, and some are damp owing to absence of eaves-spouts and damp-courses. Indeed, there are many houses in the district which might well be condemned as unfit for habitation; and the only justification for their continued occupation is absence of suitable houses into which the tenants might remove. The floors are sometimes made of bricks; sometimes they are flagged, or cemented, or boarded. Occasionally damp floors are met with. Insufficient ventilation, especially of bedrooms, is a very common defect. Some of the low attics, especially when unceiled, were very close and hot when visited by me, the summer sun having been shining on the roofs; and the high temperature thus arising from natural causes is increased, and made more oppressive, through heat ascending from the living-rooms below, in which, owing to the free coal-supply, large fires are kept burning night and day during both summer and

winter. These attics, roofed in merely by slates, afford, on the other hand, little protection against the cold of winter, and I heard of instances in which, during very cold weather, occupants were driven out of the attics and slept in the kitchens.

At the present time the most urgent matter with which the Council have to deal is the housing problem, and it is the more difficult of solution in that there are conflicting interests, the miners on one side and the colliery companies on the other. Both parties are represented on the Council. The difficulty of the position is this:—

According to the custom of this part of the country the coal companies provide dwellings for their married miners, together with a free weekly allowance of coal. If there be insufficient houses for all the married men, the companies grant to such of them as have to find their own house accommodation an allowance of 2s. 6d. a week each, as I am informed, towards whatever rent they may have to pay, and this rent may amount to 4s. or 5s., or perhaps more, per week. It has already been stated that the pitmen's houses are mostly owned or leased by the companies, so, if these are insufficient in number for the men, each man, not housed by the company for which he works, loses several shillings a week of his pay. Further, I am told that if a miner of his own accord refuse one of a company's dwellings, preferring to live in a better house and to pay his own rent, the company will not allow him the weekly 2s. 6d. towards the rent. Thus, in either event there is inducement for the miner to put up with the usual pit-village dwelling, as he is bound to be a loser otherwise. On the other hand, the coal companies would be heavy losers if private enterprise provided better dwellings, and the miners moved into these houses, and if the companies had to pay such men increased wages when they no longer housed them: for then there would be the total increase of wages so paid, plus the loss on unoccupied houses, many of them being held on lease, for which the companies would have to continue to pay rent. The miners complain of their dwellings, and this is not surprising, for, apart from the unsatisfactory character of the houses in many cases, there are not sufficient rooms for the proper housing of growing families of both sexes. The "black-men," as they are sometimes called, when they return from their work have usually to wash themselves, coal-dust begrimed from head to foot, as best they can in front of the living-room fire—yet they cannot move into better houses, as another class of workmen might, for they would get no rent allowance; or, if they did, it would be in each case a sum smaller than the actual rent.

The value of house and coals has been agreed upon between owners and miners throughout the county, for the purposes of the Compensation Act, as 5s. a week, viz., house 3s., and coal 2s. The amount paid in cash, in lieu of house, to married men varies in different parts of the county, but the

average for the whole of the county of Durham is 2s. 4½d. a week. This provision of house and coal to married men must be regarded as over and above their actual wages, and in this they have advantage over single men doing a like amount of work per week. If the miner were free to elect either to reside in a colliery company's cottage "rent free," or to live in a better-class house of his own choosing, and to draw from the coal company an extra 3s. a week, he might choose the latter course, and private enterprise might be expected to provide dwellings of a better class, so that in course of time the old pit-villages would gradually be supplanted by modern villages composed of better houses; but the coal companies would be heavy losers, as already stated. It is easy, therefore, to understand that certain of the companies are making great efforts to improve the housing, and are spending very large sums on sanitary improvements, hoping thus to satisfy the County and District Councils, and to avoid the adoption of Part III. of the Housing of the Working Classes Act, 1890, and the erection, on a large scale, of houses by the District Council. The Vice-Chairman, Mr. T. Richardson, who represents the miners, is in favour of the latter course; but the Chairman, Mr. T. F. Brass, J.P., who is a colliery manager and represents the owners' interests, is strongly in favour of miners living in the colliery companies' dwellings.

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## EBBW VALE URBAN DISTRICT

(Extracted from a Report by DR. R. FARRAR).

In respect of house accommodation, the district is in a transitional state; a considerable amount of squalid and insanitary property still exists, but a large number of well-planned and well-built artisans' houses have been erected during recent years, and the rapidly increasing supply of these bids fair to overtake the demand. It may be conjectured that in five years' time, when certain leases have fallen in and the houses held under them have been replaced, as contemplated, by dwellings of modern construction, built in accordance with the District Council's bye-laws, that the housing conditions in Ebbw Vale will give little ground for unfavourable comment.

## BUILDING CLUBS.

Of the newer houses, a large number have been erected by building clubs financed by the Ebbw Vale Steel, Iron and Coal Company, and the success of these associations not only illustrates the generosity of the company and the enterprise of their workmen, but affords an interesting example of what thrift and enterprise might accomplish in other districts.

Building clubs of this type, which were first started in Ebbw Vale in 1899, are limited to a membership of 20, to evade the necessity of registration as limited liability companies. The members must be persons in the employ of the Ebbw Vale Steel, Iron and Coal Company, who advance the necessary money for building, and have also in most instances leased the sites at a very moderate ground rent, and have, on the south side of the valley, given the stone free for the labour of quarrying.

The money for building is advanced on architects' certificates by the company at 4 per cent.; rents are collected by the club secretary; all payments by the club are made by cheque, and the accounts audited by one of the company's officials; the title-deeds of each house are held by the company until such time as the house becomes by purchase the absolute property of the member renting it. Each member pays in contributions to the sinking-fund of the club not less than 3s. a week in addition to the rent of his house, usually about 6s. a week, until all liabilities are discharged and the club is wound up; but a member may at any time reduce the debt on his house by paying into the club a sum of money other than his usual subscriptions, fines, &c., and for such sums of money paid in excess of the amount stipulated by the rules of the club he receives interest at the rate of 4 per cent. Arrangements are made to enable members to tide over periods of illness.

These building clubs, of which about 17 have been formed since the movement was initiated, have enabled a large number of the employees of the company to become the owners of their own houses, and even in some instances of two or three houses. I had a conversation with a workman who was one of the pioneers of these building clubs, and who spoke warmly of the generosity in this respect of the company.

The clubs were successful from the first, and during the colliers' strike of 1900 the company dealt leniently with the members, and did not press for repayment of sums advanced. They have advanced in this way £56,600,\* of which £16,400 has been repaid, leaving about £42,000 still outstanding. In this way more than 300 cottages have been built. The company consider that they have reached high-water mark in the matter of loans, and other people are now taking the matter up.

I inspected a number of houses built by clubs financed by the company, and found them excellent examples of working-class dwellings, both in respect of design and construction. To illustrate the accommodation afforded by these houses, I may particularize those built by the Park Building Club, in the

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\* These figures are taken from the Report of the Ebbw Vale Steel, Iron and Coal Company presented to the General Meeting of Shareholders held on June 19th, 1906.

South Ward, which contain, on the ground floor, a sitting-room, front and back kitchens, a bath-room, with hot and cold water laid on, a pantry, and upstairs, three bed-rooms. These houses are rented at 6s. per week. I may also mention a row of houses in Duffryn Road, Waunlwyd, and certain houses built by the Ebbw Steel Works Club, at Tyllwyn, which combined in a high degree artistic effect with practical comfort.

In addition to building clubs financed by the Ebbw Vale Steel, Iron and Coal Company, a large building society, "The Principality," of Cardiff, is doing a considerable business in Ebbw Vale in financing workmen who desire to build their own cottages. This society advances money at 5 per cent. interest, repayments being required at the minimum rate of 15s. per month on each share of £100. On the minimum scale of repayment redemption of property is effected in 16 years and 8 months. A large number of good workmen's houses have been built by the Graig Vawr House and Land Company in Cwm. The Cwm Building Company have built 20, and are building about 70 more such houses, and the Ebbw Valley Building Company 180.\*

The Ebbw Vale Steel, Iron and Coal Company, who are the ground landlords of most of the North and South Central and a large portion of the South Wards, and own between 1,200 and 1,400 houses, have erected some good dwellings, particularly at Waunlwyd; they have sold and are selling a good deal of their older property to builders, with the proviso that the purchasers shall pull down the existing houses and erect new ones within a given time.

The Duke of Beaufort, who is the principal ground landlord of Beaufort Ward, has replaced and is replacing, as the leases fall in, some of the older and insanitary property in the Ward by well-built modern houses.

From the above-mentioned facts it will be inferred that there has been a considerable display of building activity in the district during recent years. More than 1,000 new houses have been erected, and the greater number of these are of satisfactory construction, being built in accordance with by-laws which were allowed in 1898.

On the other hand, there still exists in the district a large amount of old and insanitary property, some of which is unfit for habitation.

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\* The Ebbw Valley Building Company must be distinguished from the Ebbw Vale Steel, Iron and Coal Company, Limited. The houses built by this Building Company may be specially noticed. They are built with cement-concrete blocks. The concrete is made from slag which is ground in a crusher, and the iron extracted by an electro-magnet to be re-melted; the proportions used are, crushed slag, 4; cement, 1; sand, 1. The blocks are made 10 inches thick with a hollow space  $3\frac{1}{4}$  inches across, bridged by a bonding course. The walls being built hollow, are warmer and drier than solid brick or stone walls; the material, which has the appearance of stone, is somewhat cheaper than brick. The slag thus utilized was formerly a waste product.

## BOROUGH OF WIGAN

(Extracted from a Report by Dr. S. M. COPEMAN).

The town of Wigan possesses within its boundaries a somewhat large proportion of comparatively low-class property, much of it, both in Wigan itself and also in Pemberton, being subdivided up into a number of courts, the sanitary condition and surroundings of which leave much to be desired.

I am informed by the Borough Engineer that there are over 170 of these courts. Each consists of a number of dwellings abutting on a courtyard, which is, in some cases, approached from an adjacent street by a narrow passage under the upper floor of one of the dwellings. Of these dwellings, some have a direct entrance from the street, as well as from the courtyard. Along one side of each court, or occasionally in the centre of the open space, is an ash-pit, or one or more ash-tubs, and privies or pail-closets, with not unfrequently, in addition, hutches and other erections in which are kept rabbits, pigeons, and fowls.

The number of houses the back-yards of which are unpaved is 1,403. These yards are common to two or more houses, the fronts of which open on to paved and channelled streets.

As a particular instance may be mentioned the yard behind Nos. 1 to 11, Golborne Street (off Platt Lane), which I found to be entirely unpaved and in a filthy condition, although the weather at the time of my visit was fine. In an adjoining yard a somewhat similar condition of affairs was, in part, due to the fact that two ash-tubs only were provided for the use of the inhabitants of six houses, with the result that the contents of the tubs were overflowing over the surface of the unpaved yard. In both these yards also were pail-closets, the pails of which on occasion from want of more frequent emptying tend to become overfull. Without multiplying instances, I may state that in the course of an inspection of a number of yards in various parts of the town, I found that the yards are for the most part unpaved, and the surface consequently uneven, and often covered for some depth with accumulated filth of various descriptions, while pools of slop-water were not unfrequently to be seen filling surface depressions.

To the importance of dealing in a satisfactory manner with the gravely unsanitary condition of the numerous courts and alleys in Wigan attention has frequently been drawn in official reports.

Thus the late Sir George Buchanan stated in 1870, as has already been mentioned, that one of the chief sanitary defects of Wigan was the "extensive want of paving and channelling in small streets and private courts." During the period that has elapsed since the date of this statement, some amount of improvement has been secured, but, especially of late, difficulty has arisen owing to the fact that much of the lower-class property in the town having come into the hands of small owners of property, the dwellings in any particular court, perhaps five or six, or even more in number, may be owned by

as many different persons. Under these circumstances the Town Council have found difficulty in dealing with the condition of these courts and yards under the Public Health Acts. The only means of improving the courts and yards is, apparently, for the Council themselves to undertake the work, comprising the levelling, paving, &c. of these areas. But if they do this they are afraid that the responsibility for their subsequent upkeep would, under the provisions of their local Acts, fall on the Town Council. I understand, however, that proceedings will be taken to do what is necessary, and if the fears of the Town Council are found to be justified, they will probably seek to obtain further powers.

Elsewhere, however, under certain recent local Acts, of which the Radcliffe Tramways and Improvement Act, 1904, may be cited as an example, powers have been obtained for coping with a similar state of affairs to that with which it is desired to deal in Wigan.

Thus, under the terms of Section 78 of the Radcliffe Act, "the owner or owners of any *existing* or future courtyard or passage used in common by two or more occupiers" shall pave it with impermeable material and *keep the same in repair*, under penalty for default.

At a conference with the Health Committee, which I attended while in Wigan, I drew their attention to the provisions of that section, under which the difficulty of paving the 1,403 back-yards which, as I am informed by the Borough Surveyor, have not as yet been dealt with, would apparently be overcome. The levelling and paving of all these yards would, in my opinion, constitute a sanitary improvement of the utmost importance, and could hardly fail to exert a beneficial influence on the health of the inhabitants of those portions of the town where at present enteric fever and epidemic enteritis are specially prevalent.

In the town generally, the sanitary condition of the individual houses is also, in many instances, extremely unsatisfactory. Thus, there still remain a number of back-to-back houses, the ventilation of which is necessarily bad, and defects of eavespouting are often to be observed. In the older parts of Wigan especially, overcrowding of houses on area is prevalent, and occasionally there is much overcrowding of persons in houses. But the surroundings and the interiors of the houses are not unfrequently filthy, owing to the want of cleanliness and common decency on the part of many of the inhabitants.

It is only fair, however, to the Town Council and to the Medical Officer of Health to mention that notwithstanding the difficulties with which they have had to cope, considerable improvement has been brought about since the date of my previous visit to the town in 1892; especially in certain areas which have been entirely cleared under the provisions of the Housing of the Working Classes Act, and on some of which the Town Council have themselves erected blocks of model dwellings. In connection with these municipal dwellings,

however, the not unusual difficulty has arisen that it has not proved possible to make provision on the spot for those persons previously resident on the particular area, for the reason that whereas the rents previously paid ranged, as I am informed, from 2s. 6d. per week to possibly, in some instances, as much as 4s. per week, the rent of the Corporation houses is 4s. 10d. or 5s. per week. As was to be expected under the circumstances, the overcrowding previously existing has, in some measure, merely been transferred to another district, the people for whom it was intended to provide accommodation not having been able to pay the increased rent.

In certain instances the Medical Officer of Health has found it possible to employ an outbreak of infectious disease as a lever for the initiation of the sanitary improvement of dwelling-houses. Thus, cases of enteric fever having broken out in New Square in 1905, the owners of the property were required to provide the houses with sculleries, water-closets and yards, an improvement which has been secured by the complete removal of the adjoining "Rylance Row," which had previously been scheduled under Part II. of the Housing of the Working Classes Act, 1890; and of one in every three of the houses in New Square, owing to the impossibility of otherwise obtaining the necessary space in the rear of the buildings. A somewhat similar improvement was secured in like manner in Robinson's Yard, Lime Street, where, with the exception of a small shop, all the cottages had been built "back-to-back" with another line of cottages in their rear. By breaking openings in the party-wall dividing the two lines of dwellings from one another, the houses, back and front, have been thrown into one, and "through" ventilation has been thus secured. But with the further result that the rents have been raised from 3s. to 4s. 6d. per week.

Now and again, in the course of my inspection, I came across instances in which, even though no special pressure had been brought to bear on them, property owners had realized the desirability of acting on the representations of the Medical Officer of Health. A number of houses in Canal Street and Holt Street belonging to an adjoining colliery may be cited. Here the yards and passages have been paved and water-closets have been provided in place of privies. I learnt from the Medical Officer of Health that the improvement of this particular area had apparently had the effect of banishing enteric fever; no cases having been reported for this area since 1904, the year in which the work was carried out, although the disease was previously endemic there.

If this be so, the importance of dealing in like fashion at the earliest opportunity with the vast amount of insanitary property still in existence in various parts of Wigan is manifest, and for this reason it is most desirable that the Town Council should obtain any further powers that would enable them to deal effectually with the old-standing problem of the insanitary condition of the numerous courts and yards in Wigan.

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## ABERAYRON RURAL DISTRICT

(Extracted from a Report by DR. W. W. E. FLETCHER).

The bulk of the houses may be said to be substantially built of local stone, with roofing of flags or slates. But there are many cottages constructed largely of clay and thatched with straw resting upon gorse, or furze, which, in turn, rests upon interlaced twigs and sticks supported by the rafters. These roofs are frequently not ceiled, so that from the interior the interlaced sticks forming the foundations of the roofs are readily visible. This arrangement offers every facility for the collection of dust and dirt, cobwebs, &c., and such dwellings cannot be kept properly clean and sweet, while thorough disinfection after the presence of infectious disease is impossible, and attempt to secure it nothing but a farce. Complaint is made that ventilation of certain of these cottages is inefficient, inasmuch as the windows are not always made to open, but in the living-rooms the old-fashioned hearths with chimneys of great width at the bottom, 6 feet or more, by, perhaps, 4 feet, gradually tapering upwards to the external opening, also of considerable size, afford pretty free ventilation, especially as the doors of the cottages are very frequently left open, and fires are almost invariably kept burning on the hearths, even during the night. The floors of these cottages are frequently composed merely of the earth trodden hard, or they are in some instances flagged. Two-storey dwellings exist, built of stone up to the bearings of the joists of the first floor, above which the walls are composed mostly of clay. In the construction both of houses of stone and of the cottages described, damp-courses are not usually provided, and the ill effects of the omission of so important a defence against damp are enhanced by the absence of rain-spouting along the eaves, a defect so common in this district that it may almost be said that eaves-spouting is more frequently absent than present. Thus, the sites of dwellings are rendered damp by the rain falling from the eaves, and the moisture is drawn up the walls in consequence of the absence of damp-courses. Some dwellings were observed the internal walls of which were very damp, in spite of the great thickness of the stonework, 18 inches or 2 feet, or possibly more in some cases. Instances were observed in which additional dampness was caused by dilapidations of roofs, and especially of thatched roofs; indeed, some cottages are hardly fit for human habitation.

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## BOURNE RURAL DISTRICT

(Extracted from a Report by DR. F. ST. GEORGE MIVART).

With the exception of the more recently built cottages, none probably in this district is provided with any sort of damp course. The want of eave-spouting is conspicuous, or when such is found, downpipes, as often as not, discharge at the foot

of house walls, where the absence of suitable, or, indeed, any paving allows soakage of the discharged water into the foundations.

Where cottagers ventured to complain at all, it was generally of the dampness of their dwellings.

It is noteworthy that rheumatism in one form or another, with its grave and disabling *sequelæ*, often entailing at an early age resort to outdoor relief, is a disease met with in all parts of this district with unusual frequency.

Combined with general dampness, the absence of ventilation, commonly remarked in dwellings here, is a most serious defect. Many of the cottages have no other means of back ventilation than a small back door. Where a back window of any sort is found, it is placed commonly so as to light a rickety staircase or tiny landing giving access to two or three bedrooms, themselves unprovided with any fireplaces, and of which often only one is lit by a single window some 20 inches square. To form a second or third bedroom a portion of the front room beneath the low sloping roof, sometimes unceiled or with ceiling broken or dilapidated, is partitioned off without windows of any kind. The atmosphere in such bedrooms as these was often found to be intolerably offensive. It must be admitted that, as was complained to me, the habits of the dwellers in these places are often dirty and careless, but it is exceedingly difficult that they should be otherwise. On the other hand, it was impossible not to notice that in some of the poorest and most hopeless dwellings, housewives were striving to do their best, and their surroundings were clean and tidy to a degree surprising under the circumstances.

To summarise briefly, the general defects most often noticed in the Bourne Rural District, as regards dwelling accommodation for the poorer class, the following may be specified as the most conspicuous:—

Generally dilapidated conditions; leaky roofs; walls thin or damaged and consequently not weatherproof.

Defective ventilation owing to inadequacy of windows and absence of fireplaces in bedrooms, and to absence of means of through ventilation.

Want of paving around houses; absence of eave-spouting and downpipes or defective arrangement of such: conditions which, in combination, constitute a serious danger to health.

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## BOROUGH OF BATLEY

(Extracted from a Report by DR. SPENCER LOW).

A number of back-to-back houses are to be seen in Batley, and the Council, despite their own bye-laws, have permitted a number of houses to be built back-to-back in blocks of four. These houses usually contain one living room on the ground floor, with two bedrooms above; sometimes only one

bedroom is provided. I am informed that the Corporation consider that such dwellings, though infringing the letter of the bye-laws, comply fully with the *spirit* of the bye-laws, inasmuch as there is a ten-feet roadway between the blocks, on to which windows open from each of the rooms, thus providing a through current of air for the interior of the house. It is, however, to be regretted that the Corporation should deliberately pass plans which contravene their bye-laws, and in establishing such dangerous precedents in the Borough they may be said to be acting in a way calculated to lead the public to infer that disregard of bye-laws is a venial offence. As a result, the Council may find difficulty in taking action against individuals ignoring their building bye-laws, as, for instance, against a person erecting a building after neglecting to submit plans.

### TROWBRIDGE URBAN DISTRICT

(Extracted from a Report by DR. R. J. REECE, on the Sanitary Administration of the Trowbridge Urban District, with special reference to the appointment of an Inspector of Nuisances).

After consideration of the report of Mr. Huddart, the Board's Assistant Inspector, the Board wrote on 13th February, 1904, asking the Urban District Council to separate the offices of Inspector of Nuisances and Surveyor, and to appoint a fully qualified person to the first-mentioned office.\*

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\* Local Government Board,  
Whitehall, S.W.,  
13th February, 1904.

SIR,

I AM directed by the Local Government Board to state that they have had under consideration the Report of their Assistant Inspector, Mr. Huddart, on the arrangements for the discharge of the duties of Inspector of Nuisances in the Trowbridge Urban District.

The Board learn from that Report that Mr. Lailey has discharged the duties of Inspector of Nuisances as efficiently as circumstances have permitted, but that the demands made upon his time by the performance of his duties in the offices of Sanitary Surveyor, Highway Surveyor and Building Surveyor, which he also holds under the District Council, have been such that even with the assistance granted to him by the Council he has been unable to give to the work of Inspector of Nuisances anything approaching the amount of time needed for a satisfactory discharge of the duties of the office. Consequently systematic inspections have not been carried out, and the sanitary condition of the district has suffered thereby.

The Board understand that the Medical Officer of Health for the District and the Medical Officer of Health for the County have both expressed the view that to secure a proper performance of the duties of Inspector of Nuisances in the district it is absolutely necessary that the officer should devote the whole of his time to the duties of the office, and the Report shows that Mr. Huddart concurs in this view.

Under these circumstances the Board are not disposed to sanction a continuance of the present arrangement beyond the 27th April next, the date to which they have sanctioned Mr. Lailey's appointment as Inspector of Nuisances, and they must therefore ask the District Council to separate the office of Inspector of Nuisances from those of Sanitary Surveyor, Highway Surveyor and Engineer,

The Urban District Council, as a result of this communication from the Board, appointed for one year from 24th June, 1904, Mr. W. Strickland as Inspector of Nuisances at a salary of £80 per annum and £2 per annum as Inspector under the Petroleum Acts, and he was required to devote the whole of his time to the duties of his office. This appointment was sanctioned by the Board.

Prior to the appointment of Mr. Strickland, in the year 1903, the total emolument received by Mr. Lailey appears to have amounted to £251, of which £85 represented remuneration additional to his salary for work done in connection with the reconstruction of the sewage works. If from this sum be deducted the moiety of the salary of Inspector of Nuisances made by the County Council, viz., £40, and there be added to it the salary paid to the Assistant Surveyor, Mr. Allen, the total cost to the District Council of these two officers in their several appointments would seem to be collectively about £283 per annum.

When Mr. Strickland was appointed as Inspector of Nuisances the total salary allotted to Mr. Lailey for the various offices filled by him was not reduced by the amount of the salary he had previously received as Inspector of Nuisances, and thus under the scheme of 1904-06 Mr. Lailey received an average salary of £185, with additional remuneration of about £110 per annum\*; Mr. Allen, the Assistant Surveyor, received £72; Mr. Strickland, as Inspector of Nuisances and Inspector under the Petroleum Acts, £82; of this last salary £40 was repaid from the county funds. The cost to the District Council of these three officers in the several appointments was therefore approximately £409 per annum.

Mr. Strickland was re-appointed with the Board's concurrence in 1905, but when this latter period of office lapsed he was not again re-appointed by the Council.

The explanation given by the Council for not re-appointing Mr. Strickland is, that with the completion of the northern portion of the sewerage works, the several offices of Highway Surveyor, Building Surveyor, Sanitary Engineer, Inspector of Nuisances and of Factories and Workshops, and Inspector under the Petroleum Acts, and Assistant Surveyor, hitherto distributed among three persons, did not now collectively

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and appoint to the first mentioned an active and fully qualified person who should be required to devote the whole of his time to the duties of the office at an adequate salary, and to keep the district under constant and systematic inspection with proper records of his work.

The Board request that the District Council will consider this matter and inform them of the result.

I am, &c,

H. C. MONRO,

Assistant Secretary.

\* I am informed by the clerk that this additional remuneration to Mr. Lailey in excess of the total of his salaried offices, amounted in the year 1903 to £85, in 1904 to £110, in 1905 to £60, in 1906 to £70, and that the sum of £100 was owing to Mr. Lailey for work done in connection with the Northern Sewage Scheme.

afford employment for more than two, and that accordingly the District Council proposed, preferring retention of their oldest officers, to appoint Mr. Allen, the Assistant Surveyor, Inspector of Nuisances as well as Assistant Surveyor at a salary of £100 per annum (£80 as Inspector of Nuisances, and £20 as Assistant Surveyor); to secure to Mr. Lailey the posts of Highway Surveyor, Building Surveyor, and Sanitary Engineer, at an annual salary of £200; Mr. Strickland to be dispossessed altogether from office. Under this scheme the cost to the District Council for the two officers collectively would be £260 per annum, provided that half the salary allotted to the appointment of Inspector of Nuisances was repaid from the county funds, but to this total amount there would need to be considered together with the additional remuneration hitherto paid to Mr. Lailey which in the past three years has averaged £110 per annum.

The points now to be determined are (1) whether efficiency in administration would be affected or any real economy be practised in reducing the staff; (2) whether the work in the Surveyor's department is likely to be less in the future than in the past; and (3) whether the sanitary circumstances of the district, which required the services of a whole time Inspector of Nuisances in 1904, have so altered as to now render such whole time service unnecessary.

The best sanitary interests of a district are not served when an Inspector of Nuisances is subordinated in his work to a Surveyor: experience has shown that the best results are obtained when the appointments of Surveyor and Inspector of Nuisances are kept separate and distinct, and the latter officer works under the directions of the Medical Officer of Health.

From the figures set forth in the preceding paragraphs it will be seen that there would be no diminution of expense to the district should the Local Government Board determine to refuse sanction to the appointment of Inspector of Nuisances being held conjointly with that of Assistant Surveyor, as in such case there would be no repayment from the county funds of a moiety of the salary of the Inspector of Nuisances. Also as in times of pressure the Assistant Surveyor is to be paid for any overtime work in the Surveyor's department, the cost under the scheme now proposed by the District Council would be actually increased, while at the same time the services of the third officer would be lost to the district.

(2) The completion of the northern portion of the sewerage works, while it has in one direction diminished the work the Surveyor has had to perform, nevertheless has very materially increased it in another. Now, in addition to his other offices, Mr. Lailey has been appointed Resident Engineer for the Sewage Disposal Works, and the supervision of these extensive works must necessarily occupy a large proportion of his time. Indeed, it would seem that so far from the work of the Surveyor's Department being lessened, it has in fact been added to.

(3) It is clear from examination of the books kept in the Sanitary Department, and on inspection of the district, that before Mr. Strickland's appointment such work as was done for the most part owed its origin to complaints by inhabitants, no attempt having been made to deal with obvious nuisances nor to systematically enforce the bye-laws and regulations of the District Council. Mr. Strickland established a very complete system of record keeping in the Sanitary Department; these books show that he endeavoured, and not unsuccessfully, to improve the sanitary condition of the houses occupied by the working classes; cowsheds and slaughter houses received due supervision; special attention was given to inspection of food. My inspection of the district revealed the amount of good work done by this officer, and corroborated the statement made by the Medical Officer of Health in his Annual Report for the year 1905 to the effect that:—"The duties of the Sanitary Inspector's department have been very efficiently discharged by Mr. Strickland." The County Medical Officer of Health, who resides in Trowbridge, refers to this appointment in his Annual Report for 1904 in the following terms:—" . . . consequently a whole time officer was appointed as Inspector of Nuisances. The appointment has been more than justified, and already there is a marked improvement in the sanitary condition of the town."

When the large amount of small and old house property is taken into consideration (as instanced by the fact that 32·6 per cent. of the houses are rated under £5 per annum, and an additional 45·4 per cent. between £5-£10), and regard is had to the work necessary in the disinfection of premises invaded by infectious disease (which average 50 per annum) and to the nature and character of the trades carried on in the district, it becomes clear that there is ample work for a qualified Inspector of Nuisances, and that the efficiency of the Sanitary Department would suffer unless the whole time services of such an officer were retained.

It is unfortunately necessary to consider the circumstances which have led up to the new proposal from another aspect. The Board received on 12th June, 1906, a letter from Mr. Strickland with reference to his not being re-appointed as Inspector of Nuisances, in which he states:—"In the performance of my duties I have unfortunately come into conflict with the private interests of several members of the Council. This was unavoidable considering that there are on the Sanitary Committee three large house agents and two butchers. . . . I have been interfered with by individual members of the Council when carrying out the details of my duties."

Mr. Strickland had been dispossessed of office at the date of my visit. It was outside my instructions to make, even if it had seemed profitable to do so, inquiry how far desire for what it was hoped would prove economical, and how far other considerations may have influenced the Council in getting rid of him. But it is right that with an eye to the future I should express my opinion that an Inspector of Nuisances in Trow-

bridge cannot be expected to carry out his duties satisfactorily without risk of conflict with important individual interests in the locality. The district contains a large number of very old houses constantly falling into disrepair, and owned either by small owners who can ill-afford to do the necessary repairs or by non-resident landlords, whose property is managed by house agents. Such houses must of necessity ultimately become uninhabitable; indeed, many in the town are scarcely fit for occupation at the present time, and already it seems that certain of the inhabitants are moving out from this class of property as new houses are erected in the suburbs. Meanwhile, this old property is becoming occupied by a lower class of persons, careless of their surroundings and ignorant of the rudiments of sanitation. Areas of this sort demand the constant attention of a trained Inspector of Nuisances. In dealing with this class of property Mr. Strickland was brought into relation with house agents, and he has an entry in one of his reports calling attention to interference, by certain of the Council, with him in the execution of his duties, and asking for the support of his Authority.

Again, in the matter of food inspection he has seized or caused to be destroyed, with the consent of the owners, unsound food, and in this work he has dealt with such food on the premises of the two butchers who are members of the District Council. There is no evidence to show that such unsound food was other than must occasionally come into evidence where large numbers of animals are slaughtered for human food, nor that Mr. Strickland exceeded the powers of his office. But in examination of the reports of the Sanitary Department there is no record, until as far back as the year 1898, of any "unsound" foodstuffs having been dealt with prior to the appointment of Mr. Strickland. In 1898 the only seizure recorded was of unsound fish. Considering that at one slaughter house alone 500 pigs are killed each week, it is manifest that food inspection was not practised in any routine fashion in the district until Mr. Strickland came into office.

An honest opinion may no doubt be held that the sanitary needs of Trowbridge can be met, and diminution of expenditure in administration effected by the appointment of the Assistant Surveyor as Inspector of Nuisances also, but I am forced to the unsatisfactory conclusion that this has not been the sole factor which has been instrumental in deciding that Mr. Strickland should not be appointed for another period of office.

*Summary.*—In the foregoing report, when dealing with the general circumstances of the district, I have pointed out such defects or shortcomings as came under my notice; and the remedies for these are sufficiently obvious without further comment from me. Speaking in general terms, I should say that the urban district is adequately supplied with good water; that it has a comprehensive sewerage system; that it is free from middens and midden-privies and the collections of putrefying organic matter associated with such structures. In

these respects it contrasts favourably with some other manufacturing districts of like size. On the other hand, there is special need in the district for careful supervision of the trade in food for man, for attention to the condition of much of the house property, and for effecting improvement in the character of house drains. These matters in particular call for sustained efforts by the Officers of the District Council, acting with the support of the Council itself. In a town like Trowbridge, where the population, mainly industrial, exceeds 11,000, there is ample work to occupy the whole time services of an active and competent Inspector of Nuisances, qualified also in food inspection; such an appointment is in the best sanitary interests of the inhabitants of the urban district.

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## APPENDIX A., No. 4.

**REPORT of the OPERATIONS of the ANIMAL VACCINE ESTABLISHMENT at 95, LAMB'S CONDUIT STREET during the year 1906-07 ; by Dr. LESLIE THORNE THORNE, Director.**

During the year April 1st, 1906, to March 31st, 1907, 66 calves were vaccinated. The aggregate weight on reception at Lamb's Conduit Street of the 66 calves was 17,240 lbs. On dismissal from the station the aggregate weight was 17,970 lbs., so that during retention for vaccination purposes they gained in weight by an average of 11·06 lbs.

Of the above calves, 27 were vaccinated direct from other calves, and 39 were vaccinated from calf lymph which had been stored ; as usual, the vaccinations performed with direct lymph proved to be more successful than those performed with stored lymph.

Insertions to the number of 869 in calf-to-calf operations produced 849 vesicles, a percentage of 96·55. In the 39 calves vaccinated with stored lymph 1,207 insertions produced 907 vesicles, a percentage of 75·14.

But little difference in the results of calf-to-calf vaccination was observed, whether the lymph used was from calves vaccinated 96 hours or from calves vaccinated 120 hours previously.

## PRIMARY VACCINATIONS.

During the year 1906-7 there were performed 986 primary vaccinations, five separate insertions of lymph being made in each instance. Of the persons thus vaccinated, 507 were males and 479 were females. All but 12 primary vaccinations succeeded at the first attempt.

There were four primary vaccinations performed from calf-to-arm ; vaccination in these four persons showed five successful insertions in each case, an insertion success of 100 per cent.

## GLYCERINATED CALF LYMPH.

During the same period 982 primary vaccinations were performed with glycerinated calf lymph. Of these, one failed to return for inspection, and of the remaining 981, 826 were found to have taken in five places, 64 in four, 41 in three, 25 in two, and 13 in one place ; 12 failed completely at the first attempt.

The insertion success, therefore, was 93·42 per cent.

There were 54 cases postponed on account of their present unfittedness for vaccination, and there were 11 cases brought back after inspection on account of some abnormal condition following their vaccination ; none of these were of a serious nature.

One case vaccinated on September 18th, 1906, and inspected on September 25th, on which date the condition of the vaccination was normal, died on October 17th from the effects of erysipelas, which began to manifest itself at the seat of vaccination on October 10th. The case was investigated by one of the Board's inspectors, who reported " that the erysipelas was probably set up by introduction of septic matter into the vaccination wounds during their healing."

## APPENDIX A., No. 5.

## REPORT on the OPERATIONS of the GLYCERINATED CALF LYMPH ESTABLISHMENT, 1906-07; by Dr. F. R. BLAXALL.

The amount of glycerinated calf lymph despatched during the year ended March 31st, 1907, was 551,750 capillary tubes, each containing sufficient lymph for the vaccination of one case.

Particulars of this despatch are given in Table I.

The results of the use of the lymph during the year for the 506,778 cases, of which report has been received, show 99·0 per cent. case success, and 94·7 per cent. insertion success.

The primary vaccinations numbered 491,685, giving a case success of 99·0 per cent., and an insertion success of 94·7 per cent.

The re-vaccinations numbered 15,093, giving a case success of 97·2 per cent., and an insertion success of 92·7 per cent.

Table II. shows the results for the whole year, arranged in quarterly periods.

The lymph thus issued was derived from the vaccination of 385 calves.

All these animals, after slaughter, were examined by our veterinary surgeon and certified to be healthy.

Two other calves, which appeared to be in health during the course of vaccination, were found at the autopsy to exhibit evidence of tubercular lesions. None of the lymph from these calves was issued.

The bacteriological examinations of the lymph have been carried out as heretofore.

TABLE I.

Showing NUMBER of CHARGES of GLYCERINATED CALF LYMPH sent from LABORATORIES to NATIONAL VACCINE ESTABLISHMENT for DISTRIBUTION to PUBLIC VACCINATORS during the year ended 31st March, 1907.

Monthly Number of Charges.	Quarterly Number of Charges.	Monthly Number of Charges.	Quarterly Number of Charges.
1906.		1906..	
April 42,000 ...	1906, Second Quarter, 147,700	October 48,900 ...	1906, Fourth Quarter, 126,950
May 54,800 ...		November 45,200 ...	
June 50,900 ...		December 32,850 ...	
		1907.	
July 46,400 ...	1906, Third Quarter, 138,450	January 47,050 ...	1907, First Quarter, 138,650
August 45,450 ...		February 43,000 ...	
September 46,600 ...		March 48,600 ...	
		551,750	551,750

TABLE II.

Showing RESULTS, of the USE of GLYCERINATED CALF LYMPH issued during the year ended 31st March, 1907.

## TOTAL CASES.

Period during which sent out.	Number of cases used for.	Percentage success.	
		Case.	Insertion.
June Quarter 1906 ... ..	136,337	99·1	95·0
September " " ... ..	129,787	99·0	94·4
December " " ... ..	112,484	98·7	93·9
March " 1907 ... ..	128,170	99·1	95·3
Total ... ..	506,778	99·0	94·7

## PRIMARY CASES.

Period during which sent out.	Number of cases used for.	Percentage success.	
		Case.	Insertion.
June Quarter 1906 ... ..	131,949	99·1	95·1
September " " ... ..	126,183	99·0	94·5
December " " ... ..	109,054	98·7	94·0
March " 1907 ... ..	124,499	99·2	95·3
Total ... ..	491,685	99·0	94·7

## RE-VACCINATIONS.

Period during which sent out.	Number of cases used for.	Percentage success.	
		Case.	Insertion.
June Quarter 1906 ... ..	4,383	98·0	93·6
September " " ... ..	3,604	96·7	91·8
December " " ... ..	3,430	96·8	92·3
March " 1907 ... ..	3,671	97·1	92·9
Total ... ..	15,093	97·2	92·7

## APPENDIX A., No. 6.

## DIGEST of the VACCINATION OFFICERS' RETURNS with regard to Children whose Births were registered in the Year 1905.

The following is a summary of the thirty-third annual return under the Vaccination Act, 1871 :—Of 929,540 births returned to the Board by the several vaccination officers in England and Wales as registered during the year 1905, the number which, at the time the return was made, had been registered as successfully vaccinated was 705,040 (being 75·8 per cent. of the whole), and the number registered as having died unvaccinated was 84,712 (or 9·1 per cent. of the whole). Of the remaining 139,788 children, 2,252 (or 0·2 per cent. of the whole) had been registered as insusceptible of vaccination ; 8 as having contracted small-pox ; 13,175 (or 1·4 per cent.) as having their vaccination postponed by medical certificate ; and 44,369 (or 4·8 per cent.) in respect of whom certificates of conscientious objection were received ; leaving 79,984 (or 8·6 per cent.) as “ removed,” “ not to be traced,” or otherwise unaccounted for. If from the 929,540 births returned by these officers deduction be first made of the deaths that took place before vaccination, it appears that, of the surviving 844,828 children, there were registered at the time of the return 83·5 per cent. as successfully vaccinated ; 0·3 per cent. as either insusceptible of vaccination, or as having had small-pox ; 1·6 per cent. as under medical certificate of postponement ; and 5·3 per cent. in respect of whom certificates of conscientious objection to vaccination had been obtained ; leaving 9·5 per cent. as at that time still unaccounted for as regards vaccination.

The proportion of cases unaccounted for in the metropolitan returns for 1905 is 18·9 per cent. ; in the provincial returns 8·6. Of the registered births of the thirty-four years, 1872–1905, the

proportion not finally accounted for in regard to vaccination (including cases postponed) in each year respectively has been as follows :—

—	Metropolis.	Rest of England.	—	Metropolis.	Rest of England.
1872 ...	8·8	4·5	1889 ...	11·6	9·6
1873 ...	8·7	4·2	1890 ...	13·9	10·9
1874 ...	8·8	4·1	1891 ...	16·4	12·9
1875 ...	9·3	3·8	1892 ...	18·4	14·3
1876 ...	6·5	4·0	1893 ...	18·2	15·7
1877 ...	7·1	4·1	1894 ...	20·6	19·0
1878 ...	7·1	4·3	1895 ...	24·9	19·8
1879 ...	7·8	4·5	1896 ...	26·4	22·3
1880 ...	7·0	4·5	1897 ...	29·1	21·6
1881 ...	5·7	4·3	1898 ...	33·0	19·6
1882 ...	6·6	4·5	1899 ...	27·7	15·4
1883 ...	6·5	4·9	1900 ...	25·8	13·9
1884 ...	6·8	5·3	1901 ...	24·1	11·2
1885 ...	7·0	5·5	1902 ...	21·3	10·0
1886 ...	7·8	6·1	1903 ...	20·7	9·1
1887 ...	9·0	6·7	1904 ...	19·1	8·7
1888 ...	10·3	8·2	1905 ...	18·9	8·6

In 1905 the proportion of cases unaccounted for (excluding the postponed cases) in the Metropolis and in the rest of England was 17·8 and 7·2 per cent. respectively.

	Births.	Successfully vaccinated.	In susceptible of vaccination.	Had Small-pox.	Number in respect of whom Certificates of Consentious Objection have been received.	Died unvaccinated.	Vaccination postponed.	Remaining.
<b>ENGLAND AND WALES.</b>	929,540	705,040	2,252	8	44,869	84,712	13,175	79,984
Ditto (excluding Metropolitan Unions).	802,898	615,539	1,956	8	42,910	73,266	11,780	57,439
<b>METROPOLITAN UNIONS.</b>	126,642	89,501	296	—	1,459	11,446	1,395	22,545
<b>REGISTRATION DIVISIONS.</b>								
London ...	126,642	89,501	296	—	1,459	11,446	1,395	22,545
South Eastern ...	83,787	68,113	198	—	4,206	5,329	1,011	4,430
South Midland ...	60,169	42,280	140	—	7,308	4,276	544	5,611
Eastern ...	54,437	38,340	84	—	3,284	4,516	666	7,547
South Western ...	44,871	34,715	78	1	3,431	3,344	980	2,322
West Midland ...	107,788	84,769	279	3	4,318	10,089	1,551	6,779
North Midland ...	59,781	35,928	73	—	7,678	5,959	934	9,209
North Western ...	151,850	120,486	447	—	5,363	15,275	2,145	8,134
York ...	101,250	79,642	394	2	4,226	9,963	1,175	5,848
Northern ...	72,075	56,911	198	1	2,167	7,468	1,337	3,993
Welsh † ...	66,900	51,355	65	1	929	6,547	1,437	3,566
<b>REGISTRATION COUNTIES.</b>								
<b>ENGLAND :</b>								
Bedford ...	4,094	2,013	5	—	1,527	281	54	214
Berks. ...	6,843	5,108	16	—	278	459	122	860
Bucks. ...	4,410	2,936	11	—	927	268	12	256
Cambridge ...	4,905	4,214	13	—	223	306	46	103
Chester ...	21,470	18,405	63	—	219	1,845	284	654
Cornwall ...	6,974	5,702	3	—	273	568	159	269
Cumberland ...	7,257	5,851	7	—	346	665	215	173
Derby ...	14,642	8,166	10	—	953	1,386	276	3,851
Devon ...	15,683	13,253	34	1	341	1,263	272	469
Dorset ...	4,749	3,784	4	—	315	323	90	233
Durham ...	42,976	33,129	123	—	1,332	4,673	808	2,911
Essex ...	33,442	22,709	55	—	936	2,719	470	6,553
Gloucester ...	16,875	10,734	56	—	2,000	1,444	496	2,145
Hereford ...	2,528	2,171	2	—	60	172	38	85
Hertford ...	7,685	5,944	15	—	549	482	68	637
Huntingdon ...	1,079	934	—	—	52	76	5	12
Kent (extra-metro.)	24,053	20,245	51	—	896	1,751	240	870
Lancaster ...	130,380	102,081	384	—	5,144	13,430	1,861	7,486
Leicester ...	12,398	3,580	8	—	3,777	1,375	322	3,336
Lincoln ...	13,288	9,645	17	—	1,742	1,251	164	469
Middlesex (ex-met.)	25,201	18,862	84	—	442	1,910	265	3,638
Monmouth... †	11,754	8,547	19	—	570	1,197	491	930
Norfolk ...	11,775	8,403	20	—	1,768	1,078	120	386
Northampton ...	8,506	4,077	5	—	3,070	695	57	602
Northumberland ...	20,472	16,716	64	—	466	2,046	295	885
Nottingham ...	19,008	14,147	35	—	1,194	1,913	169	1,550

\* The figures in this column have no reference to the other columns

† Monmouth is included in the

PERCENTAGES of BIRTHS registered in 1905.				Total Number of Certificates of Successful Primary Vaccination at ALL AGES received during the calendar year 1905.	
Successfully vaccinated.	Exempted by "Conscientious Objection" Certificates.	Not finally accounted for, including cases postponed.	Unvaccinated.		
(1)	(2)	(3)	(Cols. 2 & 3)		
75.8	4.8	10.0	14.8	709,658	ENGLAND AND WALES.
76.7	5.3	8.6	13.9	617,744	Ditto (excluding Metropolitan Unions).
70.7	1.2	18.9	20.1	91,914	METROPOLITAN UNIONS.
					REGISTRATION DIVISIONS.
70.7	1.2	18.9	20.1	91,914	London.
81.3	5.0	6.5	11.5	68,169	South Eastern.
70.3	12.1	10.2	22.3	43,246	South Midland.
70.4	6.0	15.1	21.1	39,088	Eastern.
77.4	7.6	7.4	15.0	35,039	South Western.
78.6	4.0	7.7	11.7	86,339	West Midland.
60.1	12.8	17.0	29.8	35,354	North Midland.
79.3	3.5	6.8	10.3	119,805	North Western.
78.7	4.2	6.9	11.1	79,692	York.
79.0	3.0	7.4	10.4	57,411	Northern.
81.2	1.4	7.5	8.9	53,601	Welsh.†
					REGISTRATION COUNTIES.
					ENGLAND:
49.2	37.3	6.5	43.8	2,894	Bedford.
74.6	4.1	14.4	18.5	4,857	Berks.
66.6	21.0	6.1	27.1	2,883	Bucks.
85.9	4.5	3.0	7.5	4,186	Cambridge.
85.7	1.0	4.4	5.4	18,831	Chester.
81.8	3.9	6.1	10.0	5,905	Cornwall.
80.6	4.8	5.3	10.1	5,694	Cumberland.
55.8	6.5	28.2	34.7	7,500	Derby.
84.8	2.2	4.7	6.9	13,610	Devon.
79.7	6.6	6.8	13.4	3,710	Dorset.
77.1	3.1	8.7	11.8	34,332	Durham.
67.9	2.8	21.0	23.8	23,525	Essex.
63.6	11.9	15.7	27.6	11,242	Gloucester.
85.9	2.4	4.9	7.3	2,176	Hereford.
77.3	7.1	9.0	16.1	5,770	Hertford.
86.6	4.8	1.6	6.4	970	Huntingdon.
84.2	3.7	4.6	8.3	20,005	Kent (extra-metro.).
78.3	3.9	7.2	11.1	100,974	Lancaster.
28.9	30.5	29.5	60.0	3,812	Leicester.
72.6	13.1	4.8	17.9	9,567	Lincoln.
74.8	1.8	15.5	17.3	19,550	Middlesex (ex.-met.)
72.7	4.8	12.1	16.9	8,431	Monmouth.
71.4	15.0	4.3	19.3	8,210	Norfolk.
47.9	36.1	7.7	43.8	4,268	Northampton.
81.7	2.3	5.8	8.1	16,135	Northumberland.
74.4	6.3	9.0	15.3	14,092	Nottingham.

in the table, which strictly refer to the births registered in 1905.  
Welsh Registration Division.

	Births.	Successfully vaccinated.	In susceptible of vaccination.	Had Small-pox.	Number in respect of whom Certificates of Conscientious Objection have been received.	Died unvaccinated.	Vaccination postponed.	Remaining.
<b>REGISTRATION COUNTIES—cont.</b>								
<b>ENGLAND—cont.</b>								
Oxford ... ..	4,279	3,300	7	—	518	258	37	159
Rutland ... ..	445	390	3	—	12	34	3	3
Salop. ... ..	6,691	5,790	10	—	115	482	95	199
Somerset ... ..	10,911	8,007	26	—	971	768	291	848
Southampton ...	20,160	17,420	54	—	535	1,417	170	564
Stafford ... ..	35,536	28,856	62	—	793	3,621	420	1,784
Suffolk ... ..	9,220	7,228	9	—	580	719	76	608
Surrey (extra-met.)	19,402	15,861	55	—	779	1,278	261	1,168
Sussex ... ..	13,329	9,479	22	—	1,718	924	218	968
Warwick ... ..	26,878	20,796	92	3	967	2,754	292	1,974
Westmorland ...	1,370	1,215	4	1	23	84	19	24
Wilts. ... ..	6,604	3,969	11	—	1,531	422	168	503
Worcester ... ..	19,280	16,422	57	—	333	1,616	210	592
York, East Riding	13,364	11,100	15	—	176	1,294	107	672
York, North Riding	11,244	8,611	22	—	497	1,140	223	751
York, West Riding	76,642	59,931	357	2	3,553	7,529	846	4,425
<b>WALES :</b>								
Anglesey ... ..	844	747	—	—	1	73	8	15
Brecknock ... ..	1,523	1,259	2	—	30	132	35	65
Cardigan ... ..	1,269	1,093	—	—	12	112	22	30
Carmarthen ... ..	4,254	3,786	—	—	6	379	49	34
Carnarvon ... ..	3,357	2,944	1	—	14	330	29	39
Denbigh ... ..	3,026	2,655	8	—	16	240	37	70
Flint ... ..	2,521	2,135	3	—	3	220	88	72
Glamorgan ... ..	32,517	26,257	30	1	227	3,392	560	2,050
Merioneth ... ..	1,595	1,378	—	—	9	161	23	29
Montgomery ... ..	1,557	1,255	1	—	15	140	25	121
Pembroke ... ..	2,184	1,905	1	—	8	150	56	64
Radnor ... ..	499	394	—	—	18	31	9	47

\* The figures in this column have no reference to the other columns

PERCENTAGES of BIRTHS registered in 1905.				Total Number of Certificates of Successful Primary Vaccination at ALL AGES received during the calendar year, 1905.	
Successfully vaccinated.	Exempted by "Conscientious Objection" Certificates.	Not finally accounted for, including cases postponed.	Unvaccinated.		
(1)	(2)	(3)	(Cols. 2 & 3)		
				*	REGISTRATION COUNTRIES— <i>cont.</i>
					ENGLAND— <i>cont.</i>
77·1	12·1	4·6	16·7	8,225	Oxford.
87·6	2·7	1·3	4·0	383	Rutland.
86·5	1·7	4·4	6·1	5,612	Salop.
73·4	8·9	10·4	19·3	7,854	Somerset.
86·4	2·7	3·6	6·3	18,020	Southampton.
81·2	2·2	6·2	8·4	28,906	Stafford.
78·4	6·3	7·4	13·7	7,353	Suffolk.
81·7	4·0	7·4	11·4	15,982	Surrey (extra-met.)
71·1	12·9	8·9	21·8	9,305	Sussex.
77·4	3·6	8·4	12·0	21,634	Warwick.
88·7	1·7	3·1	4·8	1,250	Westmorland.
60·1	23·2	10·2	33·4	3,960	Wilts.
85·2	2·0	4·2	6·2	16,769	Worcester.
83·1	1·3	5·8	7·1	10,880	York, East Riding.
76·6	4·4	8·7	13·1	8,674	York, North Riding.
78·2	4·6	6·9	11·5	60,133	York, West Riding.
					WALES :
88·5	0·1	2·7	2·8	786	Anglesey.
82·7	2·0	6·6	8·6	1,199	Brecknock.
86·1	0·9	4·1	5·0	1,100	Cardigan.
89·0	0·1	2·0	2·1	3,633	Cardmarthen.
87·7	0·4	2·0	2·4	2,945	Carmarvon.
87·7	0·5	3·5	4·0	2,456	Denbigh.
84·7	0·1	6·3	6·4	2,131	Flint.
80·7	0·7	8·0	8·7	26,065	Glamorgan.
86·4	0·6	3·6	4·2	1,275	Merioneth.
80·6	1·0	9·4	10·4	1,302	Montgomery.
87·2	0·4	5·5	5·9	1,865	Pembroke.
79·0	3·6	11·2	14·8	413	Radnor.

in the table, which strictly refer to the births registered in 1905.

METROPOLITAN UNIONS.	Births.	Successfully vaccinated.	In susceptible of vaccination.	Had Small-pox.	Number in respect of whom Certificates of Conscientious Objection have been received.	Died unvaccinated.	Vaccination postponed.	Remaining.
Bermondsey ... ..	4,293	3,187	9	—	22	422	9	644
Bethnal Green ... ..	4,321	1,997	2	—	20	503	43	1,751
Camberwell ... ..	6,944	4,691	15	—	90	613	81	1,454
Chelsea ... ..	1,585	1,291	3	—	8	150	33	100
Fulham ... ..	4,763	3,794	11	—	69	442	39	408
George's, St., Hanover Square.	2,220	1,945	4	—	11	174	14	72
George, St., in the East	2,060	1,504	1	—	2	198	6	349
Giles, St., & St. George	990	708	—	—	7	80	3	192
Greenwich ... ..	5,507	4,489	17	—	56	464	19	462
Hackney ... ..	6,958	4,314	13	—	90	669	49	1,823
Hammersmith ... ..	3,090	2,545	13	—	46	285	20	181
Hampstead ... ..	1,421	1,181	18	—	40	91	14	77
Holborn ... ..	4,019	2,804	5	—	25	382	48	755
Islington ... ..	8,603	6,360	28	—	110	709	66	1,330
Kensington ... ..	3,457	2,971	14	—	32	281	5	154
Lambeth ... ..	8,850	6,588	18	—	112	718	59	1,355
Lewisham ... ..	3,928	3,104	15	—	83	266	9	451
London, City of	302	227	—	—	5	29	2	39
Marylebone, St. ... ..	3,873	3,155	11	—	24	287	142	254
Mile End Old Town	4,216	1,852	2	—	11	376	57	1,918
Paddington ... ..	3,184	2,495	11	—	40	261	61	320
Pancras, St. ... ..	5,816	3,952	12	—	72	564	77	1,139
Poplar... ..	5,441	2,382	3	—	35	590	10	2,421
Shoreditch ... ..	3,896	2,027	4	—	31	434	84	1,316
Southwark ... ..	6,255	3,992	21	—	30	730	45	1,437
Stepney ... ..	1,789	1,028	—	—	8	172	12	569
Strand ... ..	187	144	3	—	—	18	5	17
Wandsworth ... ..	11,737	8,920	30	—	263	1,040	358	1,126
Westminster ... ..	539	462	—	—	3	33	1	40
Whitechapel ... ..	2,682	2,209	—	—	7	189	12	265
Woolwich ... ..	3,712	3,183	13	—	107	271	12	126
	126,612	89,501	296	—	1,459	11,446	1,395	22,545

\* The figures in this column have no reference to the other columns

PERCENTAGES of BIRTHS registered in 1905.				Total Number of Certificates of Successful Primary Vaccination at ALL AGES received during the calendar year, 1905.	METROPOLITAN UNIONS.
Successfully vaccinated.	Exempted by "Conscientious Objection" Certificates.	Not finally accounted for, including cases postponed.	Unvaccinated.		
(1)	(2)	(3)	(Cols. 2 & 3)		
74.2	0.5	15.2	15.7	3,297	Bermondsey.
46.2	0.5	41.5	42.0	2,183	Bethnal Green.
67.6	1.3	22.1	23.4	4,959	Camberwell.
81.5	0.5	8.4	8.9	1,426	Chelsea.
79.7	1.4	9.4	10.8	3,740	Fulham.
87.6	0.5	3.9	4.4	1,936	George's, St., Hanover Square.
73.0	0.1	17.2	17.3	1,692	George, St., in the East.
71.5	0.7	19.7	20.4	722	Giles, St., & St. George.
81.5	1.0	8.7	9.7	4,514	Greenwich.
62.0	1.3	26.9	28.2	4,237	Hackney.
82.4	1.5	6.5	8.0	2,517	Hammersmith.
83.1	2.8	6.4	9.2	1,239	Hampstead.
69.8	0.6	20.0	20.6	2,664	Holborn.
73.9	1.3	16.2	17.5	6,549	Islington.
85.9	0.9	4.6	5.5	2,936	Kensington.
74.4	1.3	16.0	17.3	6,612	Lambeth.
79.0	2.1	11.7	13.8	2,961	Lewisham.
75.2	1.7	13.6	15.3	244	London, City of.
81.5	0.6	10.2	10.8	3,467	Marylebone, St.
43.9	0.3	46.8	47.1	2,246	Mile End, Old Town.
78.3	1.3	12.0	13.3	2,598	Paddington.
68.0	1.2	20.9	22.1	4,053	Pancras, St.
43.8	0.6	44.7	45.3	2,375	Poplar.
52.0	0.8	35.9	36.7	2,048	Shoreditch.
63.8	0.5	23.7	24.2	4,030	Southwark.
57.5	0.4	32.5	32.9	1,054	Stepney.
77.0	—	11.8	11.8	153	Strand.
76.0	2.2	12.6	14.8	9,378	Wandsworth.
85.7	0.6	7.6	8.2	405	Westminster.
82.4	0.3	10.3	10.6	2,318	Whitechapel.
85.8	2.9	3.7	6.6	3,261	Woolwich.
70.7	1.2	18.9	20.1	91,914	

in the table, which strictly refer to the births registered in 1905.

## APPENDIX A., No. 7.

SUMMARY of the PROGRESS and DIFFUSION of PLAGUE throughout the WORLD during 1906; by Dr. R. BRUCE LOW.

## INDIA.

The total plague deaths in India recorded during 1906 numbered 356,721 as compared with 1,069,140, in 1905, and 1,112,376 in 1904. The majority of the deaths occurred during the months of March, April, and May. The subjoined table gives month by month for 1906 the number of deaths from plague reported in India :—

India.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague Deaths ..	19,612	32,423	62,411	77,328	43,870	5,868	2,855	7,939	19,350	23,901	23,801	37,362	356,721

The Punjab, Bombay Presidency, Bengal, and the United Provinces of Agra and Oudh were the chief sufferers from plague in 1906. But the annual epidemic in India was considerably smaller than in any of the four previous years, as may be seen in the appended table which gives the recorded deaths from plague in India in each of the last 11 years, 1896 to 1906 inclusive :—

India.	1896.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.	Total in 11 years
Reported— Plague Deaths ..	2,288	55,548	117,733	135,996	92,106	283,778	575,469	865,747	1,112,376	1,069,140	356,721	4,066,902

The marked diminution in the deaths from plague in 1906, no fewer than 714,770 deaths less than 1905, excited hopes that at last the fatal and persistent prevalence of the disease in India was on the wane. At the time of writing, however, it has to be admitted with regret, that the abatement which was expected has not been realised, and that 1907 bids fair to rank with the worst years of plague prevalence in India.

During 1906 a valuable report upon the investigations into plague in India was issued by the Advisory Committee, which was appointed jointly by the India Office, the Royal Society, and the

Lister Institute. Much attention has been devoted by the investigators to the study of plague amongst rats, and the modes by which the disease may be spread from rat to man, including the question of the rôle played by fleas in the propagation of plague.

The incidence of plague in each of the various Provinces and Native States of India is given below.

### BOMBAY PRESIDENCY.

(1901 Census Population, 25,468,209.)

The number of deaths from plague in the Bombay Presidency, including the native states, was 73,926, viz., 51,525 in the Presidency proper, and 22,401 in the Native States. This total is considerably less than in any of the five preceding years, for in 1905 there were 97,034 deaths; in 1904, 287,304; in 1903, 363,752; in 1902, 217,957 and in 1901, 157,552. From the weekly returns sent to the India Office by the Indian Government, it appears that 96,767 attacks were notified during 1906, though probably this understates the actual number. Calculating the case mortality on the above figures, it gives a rate of 76·3 per cent. The incidence of plague in the Presidency month by month during 1906 is shown in the subjoined table :—

Bombay Presidency.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague— Attacks ..	3,773	5,029	10,845	9,571	5,637	1,899	1,010	6,199	13,204	16,023	15,542	8,435	96,767
Deaths ..	3,210	3,892	7,386	8,061	4,586	923	913	4,421	11,499	12,766	8,079	7,571	73,926

The districts (Collectorates) which suffered most were Poona with 12,614 deaths, Kaira with 5,659, and Satara with 5,447. In the town of Poona, with a population at the 1901 census, of 120,543, there were recorded 7,319 deaths from plague, with an additional 891 deaths from the disease in the Cantonment, which in 1901 had a population of 26,546. The outbreak in Poona town began to assume epidemic proportions in August, and continued with severity till November, the height of the epidemic being reached during the last two weeks of September.

Among the general preventive measures employed in the province was inoculation by Haffkine's method, 28,394 persons receiving this protection in the Presidency (not including the Native States).

Of this number 85 at a later date were attacked by plague, and of these 39 died. Particulars, however, of these cases have not been published in the Annual Report of the Sanitary Commissioner for the Government of Bombay for 1906.

The appended table gives the number of attacks and deaths and the case mortality from plague in the Bombay Presidency,

including the Native States, during the last 11 years, 1896 to 1906 inclusive :—

Year.	Plague.		Case mortality per cent.
	Attacks.	Deaths.	
1896 ... ..	2,980	2,288	76·8
1897 ... ..	73,052	55,168	75·5
1898 ... ..	135,572	105,311	77·7
1899 ... ..	152,482	116,992	76·7
1900 ... ..	49,131	38,268	77·9
1901 ... ..	207,387	157,552	76·0
1902 ... ..	293,378	217,957	74·3
1903 ... ..	464,313	363,752	78·3
1904 ... ..	371,291	287,304	77·4
1905 ... ..	128,884	97,084	75·3
1906 ... ..	96,767	73,926	76·3
Total in 11 years ...	1,975,237	1,515,552	76·7

*City of Bombay.*—Population (census of 1906) 977,822.—The total plague attacks and deaths in the City of Bombay during 1906 numbered respectively 12,323, and 10,823 as compared with 16,308 and 14,198 in 1905. The figures for 1906 are the lowest that have been recorded since 1896, the year when plague was discovered in Bombay. The attacks and deaths from plague and the case mortality in the City since 1896, are shown in the appended table :—

City of Bombay.	1896.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.	Total in 11 years.
Plague—Cases ..	2,544	13,314	22,130	19,451	17,913	21,006	16,423	23,344	15,488	16,308	12,323	180,244
Plague—Deaths ..	1,936	11,003	18,185	15,766	13,285	18,736	13,820	20,768	13,538	14,198	10,823	152,108
Case mortality per cent ..	76·1	82·6	83·2	81·2	74·2	89·2	84·2	89·1	87·4	87·1	87·8	84·4

The epidemic of 1906 reached its height in April. Of the 10,823 fatal cases 7,238 were males and 3,585 females. The course of the 1906 plague epidemic month by month in the City of Bombay is shown in the subjoined table :—

City of Bombay.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Reported—Plague cases ..	185	708	2,877	4,507	2,856	385	173	163	180	153	71	55	12,323
Reported—Plague deaths	144	606	2,530	3,089	2,552	348	149	132	142	131	55	42	10,823

During 1906 the Medical Officer of Health and his staff carried out an amount of useful work in co-operation with the Plague Research Commission, especially in connection with rat plague

and disinfection. During the year 231,392 rats were collected and sent to the Bacteriological Laboratory at Parel. For various reasons, such as putrefaction and other conditions, a number of the rats sent could not be examined, but of the total collected 131,322 were examined, and 18,572 of them were found to be infected by plague.\*

Inoculation with Haffkine's prophylactic was employed where possible. Some 10,740 persons submitted to the operation during the year, 2,701 of that number being members of the Municipal staff, their families, or relations.

*Port of Bombay.*—During 1906 some 14,640 ships of all sorts, with 948,411 crew and passengers, were examined by the officers of the Port Sanitary Authority, compared with 14,197 vessels with 838,953 crew and passengers in 1905. At the inspection of outward and inward bound vessels, and of ships in the harbour or docks, 33 persons suffering from plague were discovered.†

The clothing and bedding of 105,302 persons (passengers and crews of outgoing and incoming ships) were disinfected. Of these 96,907 were Asiatic or African members of crews, third-class passengers or pilgrims; the remaining 8,395 were persons on board ships on which on arrival in port it was found that infectious illness had occurred. Eighty vessels were disinfected, 35 of them being outward bound pilgrim ships which were also at the same time freed from rats in most cases by means of Clayton's apparatus.

The following table gives for each of the last 10 years the number of vessels inspected at the port of Bombay; the number of persons, including passengers and crews, examined; and the number of plague cases discovered :—

Year.	Number of Vessels Examined.	Number of Crew and Passengers Inspected.	Number of Plague Cases Discovered.
1897 ... ..	72,808	1,313,117	169
1898 ... ..	71,498	1,207,571	236
1899 ... ..	65,822	1,193,339	155
1900 ... ..	65,966	1,173,050	84
1901 ... ..	34,637‡	886,310‡	73
1902 ... ..	15,432	701,964	44
1903 ... ..	14,597	719,322	77
1904 ... ..	15,154	751,039	58
1905 ... ..	14,197	838,953	27
1906 ... ..	14,640	948,411	33
	394,751	9,733,076	956

\* Annual Report of the Executive Health Officer, Municipality of Bombay, for 1906.

† *Vide* Annual Report of the Health Officer of the port of Bombay for 1906, by Lieut.-Colonel Crimmin, V.C. I.M.S.

‡ The decrease in the number of vessels and persons inspected is accounted for by the fact that vessels sailing from Bombay for ports on the coast of Kolaba district as far south as Roha, were exempt from medical inspection in accordance with the Government Notifications of April 17th and September 20th, 1901.

**THE BENGAL PRESIDENCY.**  
(Population, 1901, 49,891,164.)

There was a marked diminution in the mortality from plague during 1906 in this province—59,619 deaths having been certified, compared with 126,084 in 1905. The majority of the plague deaths, namely, 50,029, occurred in the districts of the Patna division, the two districts yielding the largest number of fatal cases being Saran and Patna with 15,534 and 14,869 deaths respectively. The greatest prevalence of the disease was from January to April, as may be seen in the appended table, which gives, month by month, the number of deaths from plague certified during 1906 in Bengal.

Bengal.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Deaths .. ..	5,434	10,346	19,855	16,532	1,761	338	102	431	506	518	906	2,833	59,619

Of these deaths 26,005 were in males and 33,614 in females. The use of prophylactic inoculation by Haffkine's method has unfortunately become unpopular in the province, only 1,777 inoculations having been performed during 1906. No case of plague was reported\* among the inoculated. The measures more generally resorted to were disinfection, and evacuation of infected houses. The destruction of rats was also very largely carried out; and to this measure some have attributed the diminished plague mortality of 1906 in Bengal.

*City of Calcutta* (1901 census, population, 847,796).—During 1906 there were registered in Calcutta 2,606 deaths from plague, compared with 7,372 in 1905, and 4,689 in 1904. The distribution of the plague deaths month by month throughout 1906 is shown in the appended table :—

Calcutta.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Deaths ..	118	133	658	971	310	149	42	45	3	42	54	53	2,606

Of this total 1,947 were males and 659 females.†

Plague has continued to manifest itself in Calcutta since 1898, though not in such large amount as at Bombay. The subjoined

\* Annual Report of the Sanitary Commissioner for Bengal for the year 1906, by Lieut-Colonel F. C. Clarkson, I.M.S.

† At the 1901 census the population comprised 562,596 males and 285,200 females.

table shows for each year, from 1898 to 1906 inclusive, the number of deaths registered in the city from plague :—

Calcutta.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Plague—Deaths ...	192	2,332	8,354	7,883	7,278	8,222	4,689	7,372	2,606

MADRAS PRESIDENCY.

(Population, 1901 census, 42,397,522.)

In 1906 there was a considerable diminution in the number of deaths from plague in this Presidency as compared with the average of the last five years. Only 898 deaths were recorded during 1906, against 5,788 in 1905, and 20,129 in 1904. The number of notified attacks was 1,334,\* and the case mortality calculated on these figures was 67·3 per cent. The incidence of fatal plague month by month during 1906 is shown in the subjoined tabular statement :—

Madras Presidency.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Deaths ..	207	132	113	93	22	13	21	36	77	64	46	74	898

Of the total 898, the deaths in males numbered 464 and in females 434.

The districts which chiefly suffered were Bellary (325 deaths), South Canara (153 deaths), and Malabar (92 deaths).

The principal preventive measures employed in the Presidency comprised (1) segregation of the sick and the contacts ; (2) disinfection of invaded houses and the personal effects of patients ; (3) evacuation of infected areas ; (4) passporting of persons arriving from infected places, and keeping them under surveillance for 10 days ; (5) destruction of rats.

It is worthy of mention that no deaths from plague occurred among persons who had been previously inoculated with the anti-plague prophylactic.†

The incidence of plague in the Presidency during the last nine years, 1898 to 1906 inclusive, is shown in the appended table :—

Madras Presidency.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Plague—Attacks ...	711	2,480	915	3,686	15,425	16,863	26,990	7,983	1,334
Deaths ...	577	1,855	685	3,035	12,603	13,291	20,129	5,788	898
Case mortality per cent. ...	81·2	74·8	74·9	82·3	81·7	78·8	74·6	72·5	67·3

\* Summary of weekly returns sent to the India Office.

† Annual Report of the Sanitary Commissioner for the Madras Presidency for 1906.

## THE PUNJAB.

(Population, 1901 Census, 24,533,088.)

There was a very considerable diminution in the amount of plague recorded in the Punjab during 1906 compared with the average of the last four years. The total attacks notified during the year numbered 120,745, of whom 104,863 died, a case mortality of 86·8 per cent. Of the total cases and deaths 105,730 and 92,115 respectively were referred to British districts, and 15,015 and 12,748 to native states. The incidence of plague month by month during 1906 in the Punjab is shown in the following tabular statement :—

The Punjab.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague— Attacks ..	3,565	3,942	12,215	33,628	37,856	8,805	518	166	693	2,637	6,323	13,608	120,745
Deaths ..	2,346	3,590	10,392	29,599	34,305	6,078	480	123	461	1,915	5,061	10,533	104,863

The districts which chiefly suffered were Sialkot (15,184 cases and 15,024 deaths), Ludhiana (13,322 cases and 8,695 deaths), and Lahore (13,222 cases and 10,614 deaths). Among the native states Patiala was the chief sufferer (8,779 attacks and 3,231 deaths).

The principal measures employed in dealing with plague in the Punjab during 1906 were surveillance over arrivals, isolation and segregation, evacuation of invaded houses, disinfection, rat destruction, and attention to general sanitation. Inoculation by means of Haffkine's prophylactic was also urged upon the native population, but fewer inoculations were performed than in the previous year. In 1906 some 27,924 persons were inoculated, and of these six contracted plague and five of them died.\*

The progress and diffusion of plague in the Punjab since its importation in 1897 is shown in the appended table :—

The Punjab.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.	Total in 10 years.
Plague— Attacks ..	302	3,135	408	883	36,739	321,938	341,267	481,412	451,791	120,745	1,758,620
Deaths ..	175	2,019	255	591	20,998	232,571	210,097	402,960	390,233	104,863	1,355,352
Case mortality per cent. ..	57·9	64·4	62·5	66·9	57·1	69·1	61·7	83·7	86·4	86·8	77·1

## THE UNITED PROVINCES OF AGRA AND OUDH.

(Population, 1901, 47,691,782.)

During 1906 the number of deaths from plague registered in the United Provinces of Agra and Oudh was 69,660, a marked

\* Report on the Sanitary Administration of the Punjab for the year 1906, by Lieut.-Colonel C. J. Bamber, I.M.S., Sanitary Commissioner,

decrease from the figures of 1905, when 383,802 plague deaths were recorded. The plague death-rate per 1,000 of the population was 1·46, compared with 8·05 in 1905. The highest mortality from the disease was noted in the months of March and April, and the lowest in June and July. The appended table shows for the United Provinces of Agra and Oudh the number of plague deaths certified month by month during 1906 :—

United Provinces of Agra and Oudh.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Deaths .. ..	5,681	8,957	16,561	16,309	4,263	206	142	676	868	1,531	3,961	10,506	69,660*

\* These figures are those which were furnished by the District Mortuary Registrars, and differ somewhat from those reported to the Chief Plague Officer,

Of these deaths 30,898 were males and 38,771 females.

The districts which had the highest number of plague deaths were Ballia 7,725, Azamgarh 6,419, Gorakpur 4,780, Bijnor 4,268, Cawnpore 4,105, and Bara Banki 4,099.\* Very little use appears to have been made of prophylactic inoculation, for only 1,846 persons submitted to the operation in 1906, compared with 6,411 in 1905. No persons inoculated in 1906 are known to have died from plague during the year. The subjoined table shows the number of plague deaths certified in the United Provinces of Agra and Oudh during the eight years 1899 to 1906 inclusive :—

United Provinces of Agra and Oudh.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Plague deaths ...	6	185	9,778	42,442	84,499	179,082	383,802	69,660

#### EASTERN BENGAL AND ASSAM.

(Population, 1901 Census, 29,812,735.)

The plague attacks reported in this Province during 1906 numbered 82, and the deaths 74. Two districts were invaded, viz., Pabna (27 cases and 24 deaths) and Mymensingh (55 cases and 50 deaths). The cases were mostly of a pneumonic type, and consequently the case mortality was high, 90 per cent. The infection was imported from Calcutta to both districts by travellers. Personal infection was the chief mode of spread. It is stated that in 1906 there was no history, in this Province, of distribution of infection of plague by rats, nor was any connection of the outbreaks with grain discovered.†

\* Annual Report of the Sanitary Commissioner of the United Provinces of Agra and Oudh for the year 1906.

† Annual Sanitary Report of the Province of Eastern Bengal and Assam for the year 1906, by Lieut.-Colonel E. C. Hare, I.M.S., Sanitary Commissioner.

## BURMA.

(Population, 1901 Census, 10,490,624.)

During 1906 there were registered in Burma 8,637 deaths from plague, 5,223 of them in Lower Burma and 3,414 in Upper Burma. This was a considerable increase as compared with 1905, when the plague deaths numbered 3,692. The chief districts which suffered in 1906 were Rangoon in Lower Burma, and Mandalay in Upper Burma. The following table gives, month by month, the number of deaths registered from plague in the Province of Burma during 1906 :—

Burma.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague— Deaths	533	841	1,749	920	481	863	1,070	717	348	292	266	887	8,637

Of these fatal cases 5,208 were males and 3,429 were females.

In the Annual Report of the Sanitary Commissioner for 1906 Colonel W. G. King, I.M.S., makes some pertinent remarks about the methods by which plague is spread, and deprecates in the present state of our knowledge any dogmatic assertions as regards either the rat or the flea being solely responsible for the transmission of the disease, to the exclusion of other considerations.

*Rangoon* (population 252,155).—Plague continued to manifest itself in Rangoon throughout the year, but the height of the epidemic was attained in June, July, and August; in these three months 1,462, out of a total for the year of 2,974, were certified. At the port of Rangoon special inspection of shipping was carried out, 2,353 vessels with 737,167 persons on board having been examined during the year. In outgoing vessels eight plague cases were detected before sailing, and two developed after the vessel had sailed. Five other plague cases were discovered in ships lying in the harbour, while two cases were discovered on board incoming vessels. A Clayton apparatus for the destruction of ship rats has been established at Rangoon, and five steam disinfectors have been distributed to the minor ports.

## NORTH-WEST FRONTIER PROVINCE.

(Population, 1901 Census, 1,990,744.)

During 1906 44 cases of plague were reported in this Province, 41 of them fatal. Of these, 41 attacks (40 fatal) were referred to the Hazara district, two to Peshawar (one fatal), and one non-fatal case to Dera-Ismail-Khan. The infection was carried to the village of Ghazi in the Hazara district from Rawalpindi (Punjab) by a man suffering from the disease, who infected his family and they in turn infected their neighbours in Ghazi; the infection ultimately extended to three adjacent villages. 39 of the 41 notified cases in the Hazara district occurred in the month of March.\*

\* Report on the Sanitary Administration of the North-West Province for the year 1906, by Lieut.-Colonel A. M. Crofts, C.I.E., I.M.S., Administrative Medical Officer of the Province.

In the previous year, 1905, only three imported cases were notified in the North-West Province.

**THE MYSORE STATE.**

(Population, 1901, 5,539,399.)

In 1906 there were notified in the Native State of Mysore 5,440 cases and 4,010 deaths from plague, as compared with 7,827 attacks and 3,959 fatal cases in 1905. The appended table shows, month by month, the number of notified cases and deaths from plague in Mysore during 1906 :—

Mysore State.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906
Plague—Attacks..	540	489	498	105	54	70	168	565	643	872	985	451	5,440
Deaths ..	467	339	314	86	60	49	150	401	528	724	616	276	4,010

There has been a diminution during the last two years in the amount of plague in Mysore as may be seen in the annexed table, which gives the plague attacks and deaths and the case mortality for each of the last nine years, 1898 to 1906 :—

Year.	Attacks.	Deaths.	Case Mortality per cent.
1898 ... ..	6,569	5,335	81·2
1899 ... ..	8,836	6,742	76·3
1900 ... ..	17,008	12,987	76·4
1901 ... ..	17,121	12,602	73·6
1902 ... ..	38,811	28,278	72·9
1903 ... ..	29,405	22,291	75·8
1904 ... ..	32,949	24,196	73·4
1905 ... ..	7,827	3,959	50·6
1906 ... ..	5,440	4,010	73·7
Total for 9 years	163,966	120,400	73·4

**THE HYDERABAD STATE.**

(Population, 1901, 11,141,142.)

The number of deaths from plague in the Native State of Hyderabad during 1906 was 1,265 as compared with 10,862 in 1905 and 25,596 in 1904. The notified attacks amounted to 1,660.\* During the four months of June, July, August and September no plague cases or deaths were reported. The subjoined table shows,

\* Summary of the weekly figures sent by the Government of India to the India Office.

month by month, the number of plague attacks and deaths in the State of Hyderabad during 1906 :—

The Native State of Hyderabad.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Attacks ..	216	448	485	86	10	—	—	—	—	280	80	55	1,660
Deaths ..	185	361	318	59	7	—	—	—	—	202	74	59	1,265

Plague first invaded the Nizam's dominions in 1897 and has continued to prevail for the last 10 years, the prevalence reaching its height in 1904 and 1905. In the table appended is shown the number of attacks and deaths from plague as well as the case mortality for each of the last 10 years, 1897 to 1906, inclusive :—

Year.	Attacks.	Deaths.	Case Mortality per cent.
1897 ... ..	10	8	80·0
1898 ... ..	4,475	3,868	86·4
1899 ... ..	7,887	6,378	80·9
1900 ... ..	1,112	938	84·4
1901 ... ..	139	95	68·3
1902 ... ..	3,035	2,689	88·6
1903 ... ..	33,908	27,697	81·7
1904 ... ..	31,374	25,596	81·6
1905 ... ..	11,374	10,862	95·5
1906 ... ..	1,660	1,265	76·2
Total in 10 years	94,974	79,396	83·6

#### THE CENTRAL PROVINCES AND BERAR.

(Population, 1901, 14,627,045.)

There was an increase in the number of plague cases and deaths in the Central Provinces and Berar during 1906. The attacks notified were 21,167, and the deaths 17,614, a case mortality of 83·2 per cent. The plague cases and deaths in 1905 numbered 15,584 and 12,769 respectively. The course of the 1906 outbreak can be seen by referring to the appended table, which gives month by month the plague attacks and deaths recorded in the Central Provinces and Berar during 1906.

Central Provinces and Berar.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Attacks ..	2,485	3,056	3,741	1,297	97	—	15	898	2,428	2,344	2,056	2,050	21,167
Deaths ..	1,723	3,211	3,539	1,155	58	—	9	359	2,151	2,187	1,403	1,829	17,614

# CENTRAL INDIA.

(Population, 1901, 8,628,781.)

The plague attacks and deaths in the native states of Central India numbered respectively 16,355 and 12,055, as compared with 3,467 cases and 2,855 deaths in 1905.

The table below gives the attacks and deaths recorded month by month in Central India during 1906.

Central India.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague— Attacks ..	140	191	518	1,000	128	6	18	914	4,369	5,011	2,040	2,028	16,355
Deaths ..	112	155	499	393	60	2	18	763	3,061	3,023	2,023	1,947	12,055

Calculated on the above figures the case mortality was 73·6 per cent.

# RAJPUTANA (including Ajmer-Merwara).

(Population, 1901, 9,723,301.)

There was a considerable reduction in the amount of plague recorded in Rajputana during 1906 as compared with the previous year, for only 1,226 deaths from the disease were certified against 32,576 in 1905. The majority of the deaths from plague in 1906 were registered in March and April. Only 68 of the deaths were referred to Ajmer-Merwara.

# KASHMIR.

(Population, 1901, 2,905,578.)

The number of plague attacks in Kashmir, including the Jammu States, during 1906 was 4,602, of which 2,735 proved fatal, a case mortality of 59·4 per cent. The bulk of the cases occurred during the months of March, April and May. The plague attacks and deaths reported in 1905 were 4,148 and 2,669 respectively, and in 1904, 11,400 and 8,691.

# PERSIA.

Plague broke out in the Province of Seistan, to the south-east of Persia, in November, 1905, and continued to prevail till August, 1906. At first the outbreak was confined to some 12 villages situated on the southern side of Lake Helmund, but later on the infection was carried throughout the province. The epidemic reached its height in April. No definite details as to the approximate number of cases and deaths from plague in Seistan have been obtainable, but from a Russian source it was stated that during the five weeks ended April 19th, 920 persons were attacked, and of these 794 died from the epidemic malady. In the "Journal de St. Petersburg" for February 22nd, 1906, it is

asserted that plague was imported into Seistan by means of merchandise from India, more particularly through bundles of infected clothes. Another suggestion was that the disease was caused in the first instance by eating the flesh of cattle which had died from a form of bovine plague, locally termed Tufangi.\* Others again were of opinion that the malady had been prevalent among the nomadic tribes for a considerable time before it was recognised as plague, and that the precise source of its origin in Seistan could not be traced. A more recent theory has been put forward by Captain Kelly, I.M.S., on plague duty in Seistan. He suggests that plague infection was brought to Seistan by migrating water fowls passing from Russian territory (Astrachan) into Persia. He notes that the plague bacillus can live in the flea for eight days, and that if, as is frequent, a wild duck were to eat a recently dead rat, fleas might be transferred to the duck, while the process was going on, and carried at once on its body to Seistan. In support of this hypothesis, it is stated that the epidemic first appeared solely among the tribesmen living in the marshes, whose main pursuits are wildfowling and fishing; and the infection did not attack the agricultural population till later. Among the chief places invaded were Nassirabad (or Nassrabad), the capital of the province, Hasseinabad, Dadeh, Akber-Abbas, Bulau, Neisar, and Jangal. Many of the inhabitants of the infected area fled to other places, carrying the infection with them, notwithstanding the military cordon which had been established; indeed, plague attacked some of the soldiers forming the cordon. Matters were further complicated by famine and by the presence of a mad Mullah in the locality, who incited the populace against the authorities on the plea that there was no plague, and that measures enforced were unnecessary. Special efforts, too, were made by the Mullah to inflame the public mind against the British Consul and his medical staff, who had been actively pressing preventive inoculation upon the inhabitants, and the isolation of those attacked by plague. As a result a fanatical mob attacked the British Consulate and the dispensary attached to it, fortunately without doing much injury. Later, some 1,800 persons submitted to inoculation with Haffkine's prophylactic, and only nine of them died of plague. By the beginning of September the malady had seemingly ceased to appear in Seistan.

There was no evidence of spread of the disease by rats; indeed, it is worthy of mention that neither *Mus rattus* nor *Mus decumanus* is found in the Province of Seistan. Though these rats are wanting, fleas are said to abound in large numbers.

#### CEYLON.

No cases of plague, ship-borne or on shore, occurred in Ceylon during 1906. But in two instances suspicion arose as to ships being infected by plague, though ultimately the diagnosis was disproved. The same precautions that have been taken for some years back against imported plague were continued during 1906.

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\* Tufangi is now said to be anthrax.

SIAM.

*Puket.*—Plague has recurred annually for the last few years in Puket, the population of which is estimated at 179,631. During 1906 the fatal cases of plague numbered 29. Little information, however, has been available as to the circumstances under which these deaths occurred.

FRENCH INDO-CHINA.

*Cochin China.*—During 1906 plague was reported at Saigon and at Bentré, but only 9 cases were officially notified. It is, however, certain that a great number of cases were concealed by the native population.

*Tonkin.*—The principal centres of plague in Tonkin were Hanoi and Vinh-yen. The first cases were noted in February, and the epidemic continued during March and April, gradually subsiding in May. Some 231 cases came under observation officially.

*Quang-Tchéou-Wan.*—The plague epidemic of 1906 in Quang-Tchéou-Wan began in March, and during its course about 2,500 persons were attacked, of whom 804 died.

STRAITS SETTLEMENTS.

(Estimated 1905 population, 603,460.)

*Singapore.*—During 1906 imported cases of plague to the number of 9 were reported at Singapore—1 in January, 1 in February, 1 in September, 2 in November, and 1 in December. This is a considerably smaller number than was notified in 1905, namely, 21.

*Wellesley Province.*—In April there was an outbreak of plague in this province, but details of it are awaiting.

FEDERATED MALAY STATES.

(Population, 1906, 915,000.)

*State of Selangor* (population, 1906, 283,619).—Three cases of plague, 2 of them fatal, were reported from Kuala Kuba, and 1 case from Kuala Lumpur. In none of these instances was the source of the infection traced.

THE PHILIPPINES.

During 1906 plague did not show itself to any great extent in the Philippines. Only Manila was affected, and that only to a slight extent.

*Manila* (population, 1903, 219,941).—Some 32 cases and 20 deaths from plague occurred in Manila during 1906. Eight cases and seven deaths were referred to the first 4 months of the year;

the remainder, namely, 24 cases and 13 deaths, were comprised in an unfortunate outbreak which occurred in the Bilibid Prison, where, owing to the threatened appearance of cholera, the prisoners were inoculated in November with anti-cholera prophylactic fluid. By some mischance a portion of this fluid became mixed up with some plague cultures with which experiments had been made in the Bacteriological laboratory, and a number of the prisoners were thus inoculated with contaminated fluid, 24 contracting plague, of whom 13 died.

One ship-borne case occurred in the month of March. The dead body of the patient, who was taken ill on board the s.s. *China* from Yokohama and Kobe, was landed; and bacterioscopic examination showed the malady, from which he died, to have been plague. Four of the crew on examination were found to have their temperature elevated and they were isolated as suspects. There was no extension of the disease beyond the above-mentioned cases.

Examination of rats was carried out systematically during the year 1906 as well as during 1905. For 12 months no infected animals were found, but in March, 1906, in one of the districts of the city some infected rats were discovered, and others continued to be found up to the end of June. But there was no coincident development of plague in man. For, in January 3 human cases and 2 deaths were reported; in February, 2 cases and 2 deaths; in March 1 fatal case, and in April 2 fatal cases. After this, though during April, May and June infected rodents were being detected, no human cases followed until November when the outbreak from contaminated inoculation fluid in the Bilibid Prison occurred.

The appended tabular statement shows the number of reported cases and deaths in Manila during the 6 years 1901 to 1906 inclusive:—

Manila.	1901.	1902.	1903.	1904.	1905.	1906.
Plague—						
Cases ... ..	508	9	218	65	45	32
Deaths ... ..	413	9	179	55	43	20

### CHINA.

*Hong Kong* (population, 329,038).—According to the official reports to the Colonial Office, there were in 1906 some 893 cases and 842 deaths from plague in Hong Kong, a considerable increase over the two previous years; in 1905 the plague deaths numbered 287, and in 1904, 495. The total plague deaths given in the Colonial report, viz., 842, is slightly larger than the aggregate of the reported weekly numbers sent to the Colonial Office by the Governor. These weekly reports give a total of 834 deaths. Probably some later cases unrecognised at the time the weekly report was sent off have been added to the aggregate.

The distribution, month by month, of these 893 attacks in Hong Kong during 1906 is given in the appended table :—

Hong Kong.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague—Attacks ..	4	23	68	165	402	175	10	8	—	1	1	—	893

Of the 893 cases no fewer than 870 were Chinese. The epidemic of 1906 followed a similar course to those of previous years, the months of April, May, and June showing the largest number of cases and deaths. (The table appended below is extended from one furnished by Dr. Mitford Atkinson, the Principal Civil Medical Officer for Hong Kong, in a paper read by him before the Section of State Medicine at the Annual Meeting of the British Medical Association at Toronto in 1906.)

TABLE showing the NUMBER of PLAGUE ATTACKS known to have occurred in the COLONY OF HONG KONG, month by month, during the 12 years, 1895 to 1906 inclusive.

Month.	1895.	1896.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
January ..	—	49	—	9	1	8	7	1	4	—	10	4
February ..	—	125	—	67	2	8	14	1	29	3	11	28
March ..	—	168	—	137	25	5	54	2	115	4	3	68
April ..	3	316	—	468	101	94	180	27	272	40	7	165
May ..	2	344	3	534	421	326	701	157	515	135	86	402
June ..	13	113	1	92	514	325	551	194	343	194	109	176
July ..	2	52	11	7	263	209	109	131	85	96	65	40
August ..	4	25	1	2	86	80	27	50	32	19	17	8
September ..	3	9	1	1	57	16	24	2	9	9	6	—
October..	—	2	—	2	4	12	1	2	5	—	4	1
November ..	5	1	2	—	1	2	1	1	4	5	3	1
December ..	12	—	2	1	11	2	2	4	2	5	6	—
	44	1,204	21	1,320	1,486	1,067	1,651	572	1,415	508*	327	893

\* The number in the Colonial Annual Report is 465. But probably Dr. Atkinson has been able to add to the figures originally given in the Annual Report.

As in former years careful watch was kept over the occurrence of plague in rats. During 1906 there were examined at the official laboratory 30,701 rats; of these 679, or 2·2 per cent., were found infected. This is a smaller proportion than was found in the 3 previous years. The percentage in 1905 was 4·7, in 1904 4·5, and in 1903 3·7.\*

\* These and other details concerning plague at Hong Kong are taken from the Annual Report on the Health and Sanitary Condition of the Colony in 1906, by Dr. J. M. Atkinson, Principal Civil Medical Officer.

The case mortality in 1906 was 94.4 per cent., a rate which suggests that all attacks did not come under official observation. In this connection it may be mentioned that according to the Annual Colonial Report for 1906 the "dumping" of dead bodies in the streets has become a common practice; no fewer than 1,447 dead bodies were abandoned in the streets during the year. A certain number of these were corpses of plague patients whose friends, fearful of the sanitary measures which would be carried out in plague-invaded houses, had abandoned the bodies in the streets to conceal their identity.

During 1906 outbreaks of plague were reported in certain Chinese seaport towns, *e.g.*, Amoy, Canton, Foochow, Swatow, and Hangchow; but no details as to these have been obtainable. The outbreak at Canton, however, appears to have been somewhat severe, as one report\* states that at the end of April from 50 to 60 plague cases were occurring daily in the town.

### JAPAN.

(Population estimated in 1901, 48,000,000.)

Plague continued to show itself in Japan during 1906, some 201 cases and 184 deaths being reported, as compared with 297 attacks and 257 deaths in the previous year. Kobe and Osaka yielded the majority of these cases. Among other places invaded were Hiroshima Ken, Wakagama, Shimonoseki, Yokohama, Sasebo, Nagasaki, Fukuoka, Jida, Yamaga, Kawaga, and Chime.

The progress of plague in Japan since 1897 is shown in the subjoined table:—

Japan.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Plague—										
Cases ... ..	1	—	62	168	3	14	58	4	297	201
Deaths ... ..	1	—	45	153	3	9	50	4	257	184

*Formosa*, 1906, population, 3,050,004. — Plague continued to manifest itself in *Formosa* during 1906. The total reported cases numbered 3,267, and the deaths 2,604, a case mortality of 79.7 per cent. The height of the epidemic of 1906 was reached in May—no fewer than 2,451 cases, and 1,909 deaths were referred to the second quarter of the year. The appended table shows the course of the epidemic month by month in *Formosa* during 1906.

Formosa.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Reported Plague—													
Cases .. ..	48	94	204	800	1,046	605	125	7	7	32	100	201	3,267
Deaths .. ..	38	68	157	602	791	516	122	12	7	23	84	184	2,604

\* Veröffentlichungen des Kaiserlichen Gesundheitsamtes for June 7th, 1906.

The chief localities affected in Formosa were the districts of Kagi, Hozan and Taipeh.

The progress and diffusion of plague in Formosa since 1896 to 1906 inclusive may be seen in the appended table :—

Years.	Plague cases.	Deaths.	Case mortality per cent.
1896	187	126	67·4
1897	730	566	77·5
1898	1,233	882	71·5
1899	2,637	1,995	75·7
1900	1,084	807	74·1
1901	4,519	3,624	80·2
1902	2,238	1,740	77·7
1903	889	709	79·8
1904	4,449	3,348	75·3
1905	2,394	2,091	87·3
1906	3,267	2,604	79·7

# AUSTRALIA.

## NEW SOUTH WALES.

(Population, 1906, 1,530,984.)

*Sydney and Suburbs* (population, estimated to 1906, 534,200).—During 1906 some 20 plague cases were reported in Sydney and suburbs, with 8 deaths. The type of the disease was mostly mild, and the number of deaths would have been less had it not been for the occurrence in June of a group of fatal pneumonic plague cases at Balmain, a suburb of Sydney. Of the 20 cases during the year, 7 occurred in March and 7 in June, 3 in July, and single cases in May, October and December. Examination of rats for plague in Sydney was continued throughout the year, infected rodents being reported in March, May, June, July, August, September, October, November and December.

The table appended shows the number of human plague cases and deaths officially reported in each of the 7 years, 1900 to 1906 inclusive.

Sydney.	1900.	1901.	1902.	1903.	1904.	1905.	1906.	Total in 7 years.
Plague—								
Cases ...	303	2	138	2	10	18	20	493
Deaths ...	108	1	38	—	6	5	8	161

In past years the greatest number of plague cases have been reported in the months of March and April.

It speaks well for the efforts of the sanitary officials of Sydney that they have been able to hold plague so successfully in check.

No other localities in New South Wales were invaded by plague in 1906.

## QUEENSLAND.

(Population, estimated to 1905, 526,369.)

During 1906 some 37 cases of plague, 16 fatal, came under observation in Queensland at Brisbane, Rockhampton and Cairns.

*Brisbane* (population, 125,500).—The 2 first cases were notified in March, 2 in April, 2 in May, 2 in June, 2 in November, and 1 in December, making in all 11 cases, of which 7 proved fatal. Systematic search for infected rats and mice was carried on, 48 infected rats being discovered during the year, out of 12,195 animals examined (10,847 rats and 1,348 mice). Of the infected rats, 16 were *Mus Decumanus*, 1 *Mus Alexandrinus* and 31 *Mus Rattus*.\*

*Rockhampton* (population, 19,691).—From April to May 11 plague cases were reported in Rockhampton, 4 of them fatal. One of the later cases was a nurse at the isolation hospital. No plague had appeared at the place for 6 years. It was locally believed that the infection in 1906 had been brought by rats, on vessels which had been moored to the town wharves. In the month of April a number of dead rats were washed up.

*Cairns* (population, 3,467).—Ten cases, only one of which proved fatal, were notified at Cairns between July and October. It is said that, though searched for, no infected rats were discovered. The disease at Cairns seems to have been of a mild type.

The appended table gives the number of notified plague cases and deaths, and the case mortality, in Queensland, during each of the 7 years, 1900 to 1906 :—

Queensland.	1900.	1901.	1902.	1903.	1904.	1905.	1906.	Total in 7 years.
Plague—								
Attacks ...	136	36	91	29	35	56	32	415
Deaths ...	57	12	33	17	12	33	12	176
Case mortality per cent. ...	41·9	33·2	36·2	58·6	34·2	58·9	37·5	42·4

## WESTERN AUSTRALIA.

There was no epidemic during 1906 in Western Australia, but three places were invaded during the year, Perth, Fremantle and Geraldton. Altogether 32 cases, with 13 deaths, came under observation.

*Perth*.—Early in January plague appeared at Perth, which lies on the river Swan, and is the capital of Western Australia. From January up to July some eight cases and two deaths had been reported, one case being referred to Subiaco, a suburb of Perth. No

\* Report on plague in Queensland 1900 to 1907, by Dr. Burnett Ham, Commissioner of Public Health.

plague-infected rats and mice were found at Perth in January and February, but later on, in March and April, infected rodents came under notice.

*Fremantle.*—Plague broke out in February at Fremantle, which is situated at the mouth of the river Swan. Up to July 14 cases and 4 deaths had been certified. The three first cases occurred in an Italian family, a father, mother and child, residing at a wine saloon and lodging house. The next one was a special constable employed outside this wine saloon, and shortly afterwards a man engaged in disinfecting the two invaded houses was attacked. Infected rats and mice were discovered at Fremantle in March and April. It is said that prior to the occurrence of the cases mentioned above two French ships from Noumea had landed amongst them five sick men from their crews, and that two of these had died in the port isolation hospital from a disease which was afterwards regarded as plague.

*Geraldton.*—Towards the end of February, 1906, several plague cases occurred at Geraldton, a seaport town, situate 290 miles north-west of Perth. Up to March 17th, 10 cases had been notified, and of these seven proved fatal. When the disease appeared search was made for infected rodents, but, it is said, with negative results.

#### VICTORIA.

*Adelaide.*—On the arrival of the s.s. *Britannia*, on July 12th, at Adelaide, from Melbourne, one of the crew was landed at the quarantine station, suffering from plague. There were no subsequent cases.

#### NEW CALEDONIA.

At the end of 1905, an outbreak of plague occurred at Noumea, and continued during January, 1906, 35 cases and 10 deaths altogether having been reported. On February 1st, the epidemic was declared to have entirely subsided.

In September two fatal plague cases were reported from La Coulée, about 12 miles from Noumea, the victims being white convicts. A military cordon was placed round the village, a campaign against rats was carried out, and all who had been in contact with the two cases were inoculated with anti-plague serum. No further cases came under notice.

#### HAWAIIAN ISLANDS.

(Population, 1898, 154,001.)

During 1906, there were reported 22 fatal cases of plague, 20 of them at Honolulu and two in Kauai. Most of the cases were Chinese or Japanese. During the last four months of the year the disease appears to have been entirely absent from these Islands.

## SOUTH AMERICA.

## BRAZIL.

(Population, 1890, 14,333,915.)

Plague has now continued to show itself in Brazil for the last seven years. During 1906, outbreaks were reported in Rio de Janeiro, Bahia, Campos, Para, Pernambuco, Sao Paulo, Guarantigueta, Port Alegre, and Nictheroy.

*Rio de Janeiro* (estimated population in 1906, 912,900).—In 1906, there were 312 attacks which came under observation, and of these 111 proved fatal, a case mortality rate of 35·6 per cent. The disease persisted throughout the year, cases being reported in each month. But the main epidemic was from September to December as may be seen by the subjoined table, which gives month by month the number of cases and deaths from plague in Rio de Janeiro during 1906:—

Rio de Janeiro.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Plague attacks	24	16	12	5	3	4	4	13	35	51	91	54	312
Plague deaths	11	4	6	1	2	1	2	7	7	15	30	25	111

Apparently the disease is becoming milder in type, for since 1900, the case mortality, as reckoned on the official figures, has become gradually less. This is shown in the appended tabular statement which shows for each of the last seven years the number of plague cases notified, the number fatal, and the case mortality per cent.:—

—	1900.	1901.	1902.	1903.	1904.	1905.	1906.	Total in 7 years.
Reported plague cases	516	384	461	792	682	353	312	3,500
Certified plague deaths	295	199	215	360	292	141	111	1,613
Case mortality per cent.	57·2	51·8	46·6	45·5	42·8	39·9	35·6	46·0

*Bahia*, properly San Salvador de Bahia (estimated population 265,000).—Next to Rio de Janeiro, the place that suffered most from plague in 1906 was Bahia, the capital of the State of that name. During the year 130 attacks and 95 deaths came under observation, giving a case mortality of 73·0 per cent. Plague had appeared in Bahia in 1904 and 1905. It is believed that the 1906 outbreak was due to a continuance of the prevalence of the two previous years, and not to a fresh importation of infection. From Bahia plague infection was carried to the Island of Itaparica, which lies across the Bay of Bahia.

*Pernambuco* (population 210,000).—From December 22nd, 1905, to October 31st, 1906, 77 plague deaths were reported in the town

of Pernambuco (otherwise known as Recife), which has an estimated population of 210,000. Details of the outbreak, however, are wanting.

*Campos* (estimated population 30,000).—An outbreak of plague occurred at Campos in August. This town is situated on a lagoon, about 30 miles from the coast line, and on the Leopoldina Railway about 2½ hours journey from Rio de Janeiro. On August 15th, 1906, a person arrived at Campos from Rio de Janeiro, and died of plague three days after arrival. Two medical men who attended him contracted the disease which proved fatal in both instances. It is worthy of mention also that a third medical man died of plague during the course of the outbreak at Campos. The precise number of attacks and deaths has not been made known, but judging by its fatality on the medical men attacked, it must have been an outbreak of some severity.

It is not possible to give details of the other manifestations of plague in Brazil (at Sao Paulo, Port Alegre, Nitheroy, Guarantigueta, and Para), as precise information with regard to these places has not been available.

#### PARAGUAY.

(Population, 1900, 535,571.)

In January, 1906, plague appeared in Asuncion, though no report was issued by the Government officials at the time. In March, however, the Paraguayan Government officially acknowledged the presence of plague in Asuncion, though no details of the outbreak, and the number of cases and deaths, were published. It was, however, admitted that the Bacteriological Institute had, after investigation, confirmed the diagnosis of plague in certain cases. Fortunately the infection did not seem to extend, and during the rest of the year no more was heard of plague in Paraguay.

#### ARGENTINA.

(Population, 1895, 4,044,911.)

Several cases of plague, some of them fatal, occurred at San Nicholas, on the river Parana during March, 1906. The disease was also reported to have appeared in the Esperanza Colony and in Roldan Colony in the Province of Santa Fé, but no details of these outbreaks have been made public by the Argentina Authorities.

During May, it was reported that plague had appeared in Buenos Ayres, and that a number of deaths suspected to have been from plague had occurred, but no official cognisance seems to have been taken of these cases, though the local newspapers stated that 12 men had at once been employed as ratcatchers in the harbour of Buenos Ayres and its neighbourhood.

Towards the end of the year reports were again circulated that plague had appeared in Buenos Ayres, but there was no official notification in confirmation or contradiction of these reports.

## CHILI.

(Population, 1895, 2,712,145.)

It is somewhat difficult to ascertain to what extent plague prevailed in Chili during 1906, no trustworthy reports being available. But from various sources information has come to hand that a number of localities, as in 1905, suffered from the disease, including Valparaiso and its suburb Vina del Mar, Antofagasta, Santiago, Taltal, Iquique, and Tacna. The importation of plague into Antofagasta was locally attributed to infected sugar sacks from Peru, but it is known that plague had already invaded Antofagasta in 1905. At Santiago the outbreak of plague began at the barracks, but there is evidence that the Chilean Authorities did their best to conceal the occurrence.

## PERU.

(Population, 1896, 4,559,550.)

Plague, which had been present in Peru during 1905, continued to prevail in a number of places during 1906. The chief localities which suffered from epidemic plague were Lima, Trujillo, Payta, and Mollendo, but other smaller outbreaks occurred at San Pedro, Pacasmayo, Lambayeque, Chiclayo, Catacas, Nuevo Chosica, Reque, Callao, Mansefu, Eten, Salaverry, and Pisco. Definite details of these outbreaks, however, have not been obtainable at the time of writing.

A death suspected to have been due to plague, occurred on board the s.s. *Palena*, which arrived at Callao on May 2nd, 1906, on its voyage from Salaverry. There had been, it was stated, an epidemic of illness among the rats on board. On October 1st a steerage passenger was landed at Lima from the s.s. *Limari* suffering from a mild attack of plague contracted it was believed at Valparaiso. During the prevalence of plague at Lima in the first four months of 1906 there was also concurrent plague among the town rats.

## TRINIDAD.

The s.s. *Indus* left Calcutta on October 30th, 1906, with 750 emigrants for Trinidad, where she arrived on December 13th. Twenty-three of the passengers had died during the voyage. The ship's surgeon had certified these deaths as due to various causes, viz., cerebrospinal meningitis, 9; broncho-pneumonia, 4; pneumonia, 3, and other causes, 7. None of the crew had had any illness. The authorities regarded these facts as raising suspicion of plague, and the ship was disinfected, after which the *Indus* sailed for Cuba. It was stated that among the passengers landed at Cuba 11 further deaths occurred during December, making a total of 34 of the *Indus's* passengers who died.\* Apparently no corroboration of the suspicions of plague was obtained, for no official intimation reached this country that any of the passengers landed from the *Indus* had suffered from a disease of the nature of plague.

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\* United States Public Health Reports for January 4th, 1907,

## UNITED STATES.

On April 6th, 1906, the s.s. *Burrsfield* from Bombay called at the Quarantine station, Reedy Island, Delaware, and reported that four cases of suspected plague had occurred among the Lascar crew; and also that two others under suspicion had died on the voyage. A bacterioscopic examination of material from the suspected cases confirmed the diagnosis of plague. Two other attacks were subsequently reported, one on April 10th and the other on April 11th, among the Lascars who had been landed from the *Burrsfield*, and that one had proved fatal. The usual precautions were taken, including fumigation of the ship, after which she continued her voyage, having embarked a new crew. There were no subsequent developments of plague in connection with this vessel.

## THE CANARY ISLANDS.

During November and December, 1906, some cases of "infectious fever," about 28 in number, 12 of which proved fatal, occurred at Santa Cruz, the capital of the island of Teneriffe. Rumours became current in December that these cases were in reality plague, and that the authorities were concealing the facts. In consequence of this stringent quarantine measures were instituted by neighbouring ports against Santa Cruz, and especially by its great rival, Las Palmas, on the island of Grand Canary, which refused to have any communication at all with Santa Cruz. For a time at least the rumours were believed, even at Madrid; and a Government expert and bacteriologist was despatched to Teneriffe to investigate the outbreak. After considerable delay the expert pronounced the cases to be enteric fever and not plague. Meanwhile the traders and the commercial classes at Santa Cruz had sustained serious pecuniary losses by the transfer of their trade to Las Palmas. Some persons even went so far as to allege that the plague rumours had been circulated purely with a view to get trade transferred to Las Palmas. Others again believed that the mischief arose from the blind panic into which people in the Canary Islands were thrown by the idea of plague being in their midst.

## MADEIRA.

*Funchal* (population, 44,000).—Towards the end of 1905 several cases of illness diagnosed as an "infectious fever" of an indefinite kind occurred in Funchal, and were removed to the lazaretto for treatment. The authorities seem to have suspected plague, and with a view of stopping its spread all cases of illness of a suspicious kind were visited and, as a precautionary measure, were removed along with their whole families to the lazaretto to be isolated there. The treatment which they experienced in this institution seems to have been of a very rough and trying description, and ultimately, as the information about it leaked out, strong indignation against the authorities, especially against those connected with the lazaretto, was aroused amongst the people of the town. This culminated in January, 1906, in a series of riots

during one of which the enraged mob (comprising about 3,000 persons, among whom were about 100 soldiers, two of whose comrades were detained in the lazaretto), forgetting their fear of infection, stormed the gates, wrecked the building, and carried off the sick and the contacts back to their homes. The Medical Officer of the lazaretto, who was blamed for having raised the suspicion of plague unnecessarily, came in for a considerable share of the odium incurred by the authorities, and had to flee for his life on board a Portuguese cruiser lying in Funchal harbour. It was afterwards officially certified that none of the suspected cases had been plague, and that most of them had been suffering from influenza. Much of the trouble was due to the attitude of the authorities, who refused to furnish information to the inhabitants of the town as to the nature of the suspected illness in question, and as a result the wildest rumours were current; some of them asserting that contacts in the lazaretto were being forcibly inoculated with plague virus to produce an epidemic in order that the Government at Lisbon might be induced to vote money to be spent at Funchal by the resident officials.

## SOUTH AFRICA.

*Cape Colony* (population, 1904 census, 2,405,552.)—There were no human cases of plague discovered in Cape Colony during 1906; the last reported case in man was discovered at Port Elizabeth on November 18th, 1905, and he was discharged from hospital on December 29th. But in several localities plague infected rats and mice were found, the diagnosis being bacterioscopically confirmed. At *Port Elizabeth* (population, 46,626) infected rats and mice were found during January, 1906; but during February, March, April, May, and June no traces of plague in rodents were found. In July infected mice were discovered at Port Elizabeth, but after that up to the end of the year no infected animals came under observation. At *East London* (population, 49,254) plague infected rats and mice were found during May, June, July, and August, but only in trifling amount, the rest of the year showing no evidence of plague infection in rodents. At *King Williams Town* (population, 103,552) infected rats and mice were found during the month of July, but at no other time during the year.

## NATAL.

(Population, 1904, exclusive of Native Areas, 284,691.)

There was no reported plague in man or animals in Natal during 1906; the last human case occurred in April, 1905, at Durban.

## BRITISH EAST AFRICA.

A few cases of plague, about 7 in number 3 of them fatal, were reported at Nairobi in the latter part of January and beginning of February, 1906. By the 12th of February the officials were able to declare Nairobi free from plague.

GERMAN EAST AFRICA.

In February, 1906, there occurred at Muansa 2 fatal cases of plague, and on board an English steamer on Lake Victoria a fatal case was notified on February 4th.

MAURITIUS.

There was an increase in the number of plague attacks and deaths recorded during 1906 in Mauritius compared with the previous year. The cases which came under observation in 1906 numbered 428, of which 289 were fatal, a case mortality of 67·5. The corresponding figures for 1905 were attacks 308, deaths 251, case mortality 81·5.

The epidemic of 1906 followed a course similar to those of the previous years, the prevalence reaching its height in late autumn or winter months. The subjoined table shows month by month the progress of plague in Mauritius in 1906 :—

Mauritius.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Recorded plague—													
Cases .. ..	10	4	2	—	—	—	2	23	62	101	150	74	428
Deaths .. ..	9	1	—	—	—	—	2	20	39	72	98	48	289

It will be seen from the above that plague practically disappeared from Mauritius during 3 months of the year. The chief localities affected in the island were Port Louis, the capital of the Colony, where the majority of cases occurred, and the district of Plaines Wilhems. Owing to the periods for which the monthly official reports are transmitted to this country, I am not able to distribute the above reported cases as to the locality and race over the calendar year, the monthly reports being dated usually from the 10th of one month to the 10th or 11th of the next month. For the period extending from January 11th, 1906, to January 10th, 1907, there were reported in the Colony 448 plague cases and 333 deaths; of these, 402 cases and 302 deaths were referred to the town of Port Louis; 36 cases and 24 deaths to the district of Plaines Wilhems; the remaining 10 cases and 7 deaths were distributed between the 4 districts of Pampelmousses, Rivière du Rempart, Moka, and Savanne. As regards the racial distribution, the subjoined tabular statement shows the number of attacks and deaths among Mauritians, Indians, and Chinese :—

Races.	Cases.	Deaths.
Mauritians, White ... ..	12	7
Mauritians, non-White ... ..	168	125
Indians ... ..	216	157
Chinese ... ..	52	44
Total, January 11th, 1906, to January 10th, 1907	448	333

During this period 103,643 rats, mice, and muskrats were destroyed; of these, 9,263 were microscopically examined for plague; 131 were found infected, and 39 others were regarded as "suspicious." In addition, 6 sick cats were examined, 1 being found plague-infected, while another was looked upon as "suspicious."

The number of plague attacks and deaths recorded in each of the 8 calendar years 1899 to 1906 inclusive is given in the appended table:—

Mauritius.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Reported plague—								
Cases ... ..	1,416	796	1,093	506	1,395	568	308	428
Deaths ... ..	1,117	593	805	386	1,035	449	251	289
Case mortality, per cent. ... ..	78·9	74·5	73·6	76·3	74·2	79·0	81·5	67·5

In 1906, as in former years, there was a good deal of concealment of cases. In the period from January 11th, 1906, to January 10th, 1907, no fewer than 141 out of the total 448 cases reported were not discovered till death had taken place—*i.e.*, 35 per cent. of the cases were not brought to official notice during life. It is more than probable that this concealment of infected cases leads to spread of the disease.

### EGYPT.

(Population, 1904, 10,498,870.)

Plague showed a considerable increase in Egypt during 1906, as compared with the previous year. The total number of reported cases was 631, of which 475 were fatal, a case mortality of 75·3 per cent. The figures for 1905 were 266 cases and 181 deaths, the case mortality being 68·0 per cent. A number of the cases during 1906 were not discovered till after death, a proof that concealment of cases was practised.

The distribution of the cases and deaths month by month throughout the year is shown in tabular form below:—

Egypt.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Reported plague—													
Cases .. ..	—	2	17	196	114	41	26	43	38	32	56	67	631
Deaths .. ..	—	1	14	162	80	31	14	31	23	23	38	50	475

Only a single case occurred at Cairo. There were outbreaks in Alexandria, Suez, and Port Said. Among the provinces the chief sufferers were Keneh, Minieh, and Guirgneh.

The appended table gives the number of plague attacks and deaths in the cities and provinces of Egypt during 1906 :—

Invaded City, Port, or Province.	Population calculated to July 1st, 1906.	Plague.		Remarks.
		Attacks.	Deaths.	
<b>CITIES &amp; PORTS.</b>				
Cairo ... ..	703,731	1	1	
Alexandria ... ..	370,533	99	61	
Port Said ... ..	60,981	14	11	
Suez ... ..	19,691	62	45	
<b>PLACES INVADIED IN THE PROVINCES.</b>				
<b>PROVINCES.</b>				
Behara ... ..	629,758	1	1	Damanhour.
Garbieh ... ..	1,448,002	18	16	Tantah.
Menoufieh ... ..	975,704	15	10	Tala, 14 cases, 9 deaths; Menouf, 1 fatal case.
Beni-Socuf ... ..	354,438	9	4	Beni-Socuf, 3 cases, 2 deaths; Beba, 5 cases, 1 death; Wasta, 1 fatal case.
Minieh ... ..	632,163	131	62	Samalont, 130 cases, 61 deaths; Minieh, 1 fatal case.
Assiout... ..	928,457	2	—	Mellawi.
Guirgueh ... ..	824,459	57	52	Guirgueh, 47 cases, 43 deaths; Tema, 9 cases, 8 deaths; Tahtu, 1 fatal case.
Keneh ... ..	825,175	222	212	Keneh, 21 cases, 20 deaths; Nag-Hamadi, 51 cases, 47 deaths; Dechneh, 149 cases, 144 deaths; Kous, 1 fatal case.
Totals* ... ..	—	631	475	

\* The total population of Egypt, estimated to 1st July, 1906, was 10,927,015; at the census of 1904 it was 10,386,423. Bedouins are not included in the estimated population. At the census of 1897 the Bedouins numbered 602,139.

Plague has persisted in Egypt, though not in any large amount, for the last 8 years. The high case mortality in 1906 appears to suggest concealment of cases, as has been already said, and this may perhaps be associated with the native anti-European movement which has been increasing in Egypt of late. The incidence of plague in Egypt during the 8 years 1899 to 1906 inclusive is shown in the subjoined tabular statement :—

Egypt.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Reported plague—								
Attacks ... ..	93	127	205	483	361	854	266	631
Deaths ... ..	45	60	102	292	159	506	188	475
Case mortality, per cent. ... ..	48·4	47·2	49·8	60·5	44·0	59·3	68·0	75·3

The measures carried out by the Egyptian Sanitary Department are very comprehensive and thorough, but the efforts of that department are much hindered by the unwillingness of the native

population in certain localities to comply with the measures prescribed for their benefit by the Director-General and his staff.

*Alexandria* (population, 309,916).—It cannot be said that this city in recent years has been a great sufferer from the ravages of plague. During 1906 only 99 cases, 61 of them fatal, were reported, the majority of them occurring between July and October, as may be seen in the statement appended :—

Alexandria.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Reported plague—													
Cases .. ..	—	1	1	—	1	10	17	24	13	15	9	8	99
Deaths .. ..	—	1	1	—	—	6	10	18	6	9	4	6	61

It is somewhat surprising that Alexandria, though having an annual recurrence of plague during the last 8 years, has not suffered from a serious epidemic of the disease. Much, however, may be due to the care and attention paid by the authorities to the dealing with detected cases promptly and effectually.

The appended table gives the number of plague attacks and deaths in Alexandria, with the case mortality, reported in each of the last 8 years 1899 to 1906 inclusive :—

Alexandria.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Reported plague—								
Attacks ... ..	93	37	52	100	129	108	127	99
Deaths ... ..	45	25	26	50	83	70	87	61
Case mortality, per cent. ... ..	48·4	67·6	50·0	50·0	64·3	64·8	68·5	61·6

*Suez*.—As in former years, a number of vessels landed at Suez cases of actual or suspected plague which had occurred on the voyage, and which were conveyed to the sanitary station at Moses Wells for isolation and treatment, as well as to enable a bacterioscopic examination to be made.

At the end of July plague broke out among the native population at Suez, and continued to manifest its presence till the beginning of November, 62 cases and 45 deaths coming under notice. From Suez the disease was carried to Port Said, when 14 persons were attacked and 11 died.

## TURKEY.

*Jeddah*.—At the end of May plague appeared at Jeddah ; up to July 30th there had been reported 75 cases, all of which proved fatal. These figures suggest that all persons attacked did not come under notice. It is known, indeed, that of the first 20 cases, 5 were not discovered till after death. It is therefore more than

probable that concealment of cases took place. From Jeddah the infection spread to other places, as, for example, in June, to Candirah, a village not far from the town. The infection was also carried to Mecca, where, however, only 2 cases, both fatal, occurred in June.

As regards the early cases at Jeddah, it is stated that those first attacked were labourers who had handled sacks of rice imported from the Port of Bombay, and it is surmised that infected rats had probably travelled inside the bags. But it is believed that plague had existed in Jeddah for some time before it was recognised.

*Koweit* (or Kūweit or Grane).—At Koweit, on the coast of Asiatic Turkey, a port in the Persian Gulf, a single plague case was landed in April from the s.s. *Dwarka* which had come from Bombay. But there was no extension of the infection from this case.

*Trebizond*.—In August plague appeared at Trebizond. Altogether 10 cases and 3 deaths were reported between August 5th and 12th. The outbreak began in the prison in the person of a vagrant arrested at Constantinople; he was sent thence to Trebizond where he arrived on July 11th. His illness is alleged to have commenced a few days after his arrival, but he was not medically examined till August 5th. He died on August 8th. On the same day 3 other prisoners were found to be ill. They had all been in association with the first case. Subsequently 3 others all of them from the same part of the prison as the previous cases were attacked with similar symptoms. Bacterioscopic examination confirmed the diagnosis of plague. It has not been ascertained how the infection was contracted by the first patient. No known cases of plague had occurred in the town of Trebizond since December, 1904, when a single suspected case was reported. The prison is said to have been overcrowded and in a filthy condition.

*Adalia*.—Towards the middle of August plague was reported to have appeared at Adalia, and from that date to October 5th some 14 cases, 4 of them fatal, came under observation. A similar outbreak occurred in the previous year during July, comprising 9 cases. But whether the occurrence of plague in Adalia in 1906 was associated with the outbreak of 1905 has not been ascertained, though it was asserted that one of the persons first attacked in 1906 had worn a dress which had belonged to a relation who died of plague in 1905, the dress having been concealed for some months for fear of its being burnt by the authorities. It is also said that some dead rats were discovered in a ditch behind the first house that was invaded by the disease in 1906. The diagnosis of plague in the earlier human cases of 1906 was confirmed by bacterioscopic investigation. It was rumoured that suspicious cases of illness resembling plague had occurred at Bondjak, 16 hours distant from Adalia, but there was no official confirmation of this rumour.

*Rhodes*.—In the *Bulletin Sanitaire Belgique* of October 4th, 1906, it was stated that a case of plague had occurred on September 20th at Rhodes in the person of a sailor on board the sailing vessel *Hifzi-Rahman*, which had left Alexandria on

August 24th. The ship was sent to the sanitary station at Clazomene, where the crew, comprising 5 men, were kept under observation till the danger had passed. The patient recovered.

*Beirut.*—In November 2 plague cases were reported from Beirut, one from a village just outside the town, and the other from the town itself. No further extension of the disease took place in 1906.

## RUSSIA.

In the early months of 1906 plague appeared in the Government of Astrachan, in the Kirghiz Steppe. The places invaded were the villages of Karabaklan and Obshorovo, and the town of Klasny-Jar, in the district of Krasnoyarsk ; also Bodan in the Maritime district. The number of cases does not appear to have been large, and the disease did not become epidemic though the type was somewhat severe. For example, in one Kirghiz family seven persons were attacked, and of these five died, apparently from pneumonic plague.

No further outbreak of the disease was heard of in Russian territory during 1906 till September, when some cases were reported in the Cossack village of Abagaitoui in the Akschine district, in the Trans-Baikal Government. From this place infection was carried in October to the village of Mandjouria by a military male nurse who fell ill on his arrival, and from him other persons, though in no great numbers, became infected. It is stated that the first person attacked at Abagaitoui became ill after eating the flesh of a Steppe marmot (*Arctomys bobac*), an animal which is extremely susceptible to a disease regarded as being identical with plague. A military cordon was placed round each of these villages, and several medical experts, including a bacteriologist, were sent at once by the Russian Government to deal with the outbreak, which happily soon subsided.

## AUSTRIA.

During May a paragraph appeared in a London newspaper to the effect that two cases of illness suspected to be plague had occurred at Kunau in Austrian Silesia. The infection it was surmised had been conveyed to that place by means of bales of goods brought from Russia. No official confirmation of these cases was issued by the Austrian Government ; so that it is doubtful if they really were cases of plague.

In November an Austrian quartermaster on board the s.s. *Calypso* (engaged in the transshipment service, between Trieste and Venice, for goods arriving from India and the Far East) was attacked by illness suspected to be plague, and bacterioscopic examination established the correctness of the diagnosis. He died of what appeared to have been pneumonic plague on November 8th at the town hospital of Trieste, whither he had been taken on November 7th.

The *Calypso* had not touched at any infected port, and had been making regular trips between Trieste and Venice. It is said that she had conveyed a quantity of jute and cotton, transhipped at Trieste from the Austrian-Lloyd steamer *Austria* which arrived at that port from India in the latter part of October. All necessary precautions were taken, and some 30 persons who had been associated with the deceased in the latter stage of his illness were kept under observation till November 19th, some of them being injected with anti-plague serum. No other cases supervened.

#### GERMANY.

There were no cases of human plague reported in the German Empire during 1906, though in December suspicion arose that four persons attacked by illness on board the s.s. *Santa Fe*, from the River Plate, were suffering from plague, another of the crew having died during the voyage. But the suspicion of plague was not confirmed by expert investigation. At the port of Hamburg, however, plague infected rats were discovered earlier in the year on board two ships both of which had arrived from ports in the River Plate.

1. The s.s. *Sevilla* arrived in the Elbe on April 2nd, and after the usual inspection was permitted to pass on to Hamburg. On the afternoon of April 2nd unloading of her cargo of wheat, linseed, hides, and wool was begun. Almost at the beginning of the process six dead rats were found, and these were at once sent to the Hygienic Institute of Hamburg for examination. The same day the report came that the rats were highly suspicious of plague. In consequence of this unloading was suspended, and the ship removed from the quay to an isolated part of the port, where fumigation by gas containing carbonic oxide for destruction of rats was carried out, after which disinfection of the crew's quarters and other portions of the ship was proceeded with. On April 4th definite confirmation of the diagnosis of plague in the dead rats was forthcoming. Altogether 642 rats were found before and after the fumigation on board the *Sevilla*; of these 24 were plague infected, 14 of whom were caught alive before the fumigation began.

It is noteworthy that no cases of sickness of a kind likely to have been plague had occurred on board this vessel during the voyage or afterwards.

2. The s.s. *Arad* arrived in the Elbe on May 17th. After being examined at Groden she passed up the river to Hamburg, where discharge of cargo (grain) was begun on the following day, May 18th. During the period between May 22nd and 26th 14 dead rats were discovered; on examination, however, at the Hygienic Institute the results were reported as quite negative with regard to plague. But on May 28th seven more rats were found, three of which on expert examination were pronounced definitely to be infected by plague. The unloading was at once stopped and the ship isolated while fumigation and disinfection were carried out. In all 144 rats were found on board before and after fumigation, 69 of them being plague infected. There had been no cases of sickness on board the ship during the voyage, or after arrival at Hamburg.

## FRANCE.

*Havre*.—A French sailor, who it is said had recently returned from Rio de Janeiro but had been employed at Havre for a fortnight by a local firm, was attacked by illness suspicious of plague on July 3rd, 1906. He was removed to the hospital, where he was seen by at least four medical men, who agreed that the case was probably one of plague. The illness proved fatal. Material was taken from the glandular swellings and submitted to bacterioscopic examination. The results were at first said to be confirmatory of plague, but later information was to the effect that the man died of acute septicæmia. As the case was not officially notified to the other European Governments, it is presumed that the evidence did not support a diagnosis of plague.

## SPAIN.

In the Paris edition of the "New York Herald" for September 21st, 1906, and also in a number of French newspapers, it was reported that a ship from Australia had arrived at Barcelona on board which several suspected plague cases had occurred. This statement was, however, immediately denied by the Health Director of the Port, as well as by the Mayor of Barcelona, and by the Civil Governor of the Province.

## ENGLAND AND WALES.

There was no actual plague in any of the ports of England and Wales during 1906, though 16 ships arrived technically infected, or having on board a case of illness suspected to be plague.

In seven of these 16 instances the suspected case was landed at the port hospital and material taken from his enlarged glands for bacterioscopic examination. In none of these instances was any evidence found to establish a diagnosis of plague. In the remaining nine cases the suspicious illness had occurred during the voyage or in the port of departure, the patient being landed at the first available port; in two fatal instances the body was buried at sea.

Of the 16 vessels in question, no fewer than 13 came from Indian ports, two from Australia, and one from Japan. As regards the ports of arrival, seven of the ships came to the Thames, six to Liverpool, and one each to Bristol, Plymouth, and Southampton.

In the subjoined table the facts as to these 16 ships are summarised :—

TABLE giving, in regard of 16 Ships which, in 1906, arrived in Ports of ENGLAND and WALES under Suspicion of being infected by PLAGUE, their Names, Dates of arrival, Ports of arrival and departure, Number of suspected cases, and the results of Bacterioscopic investigation where that had been carried out.

	Name of Vessel.	Date of Arrival.	Port of Arrival.	Port of Departure.	Number of Cases of Plague or Suspected Plague.	Result of Bacterioscopic Examination, if any.	Remarks.
1	S.S. <i>Soindia</i>	January 12th	Liverpool	Bombay	1	Negative.	Landed at Suez.
2	S.S. <i>Martaban</i>	February 3rd	...	Rangoon	1	"	
3	S.S. <i>Istria</i> ...	" 5th	The "Thames	Calcutta	1	"	
4	S.S. <i>Amaraipoora</i>	" 24th	Liverpool	Rangoon	1	"	
5	S.S. <i>Oriona</i>	March 26th	The Thames	Brisbane	1	"	Landed at Suez.
6	S.S. <i>Ionian</i>	April 23rd	Southampton	Bombay	1	Positive	Landed at Aden.
7	S.S. <i>Masilila</i>	" 23rd	Liverpool	"	1	Negative.	
8	S.S. <i>Pegus</i> ...	May 17th	"	Rangoon	1	"	Died on voyage, buried at sea, 3 days after leaving Rangoon.
9	S.S. <i>Cluny Castle</i> ...	June 22nd	The Thames	"	1	Positive	Occurred while in the port of Rangoon.
10	S.S. <i>Dongola</i>	July 24th	"	Bombay	2	1 Positive	1 died at sea, and 1 landed at Penang* on voyage from Bombay and Yokohama.
11	S.S. <i>Arabia</i>	August 11th	Plymouth	"	1	Negative.	Landed at Adelaide.
12	S.S. <i>Britannia</i>	" 18th	The Thames	Sydney	1	"	Landed at Shanghai.
13	S.S. <i>Japan</i> ...	September 6th	"	Yokohama	1	Positive	
14	S.S. <i>Cara</i> ...	October 24th	Bristol	Bombay	1	Negative.	
15	S.S. <i>Persia</i> ...	December 9th	The Thames	Calcutta	1	"	Landed at Port Said.
16	S.S. <i>Australia</i>	" 31st	Liverpool	Bombay	2	"	

\* The S.S. *Dongola* sailed from Bombay to Yokohama, and during that voyage the 2 plague cases occurred, 1 a native fireman on April 19th dying on the second day of his illness, the other being landed at the Penang Hospital on April 26th, when bacterioscopic investigation verified the diagnosis of plague. The vessel remained at Yokohama 14 days, and then sailed for England on June 3rd.

In addition to the case landed at Aden from the *Ionian*, another suspected case occurred on shore in Wales, and was brought to the knowledge of the Board on May 7th, *i.e.*, 14 days after the vessel's arrival at Southampton. But after careful investigation by Dr. Klein, the results were pronounced entirely negative as regards plague. The details of this occurrence are as follows:—The S.S. *Ionian* was a troop transport, and left Bombay on April 1st. She landed an English non-commissioned officer on April 6th at Aden, suffering from plague. At Suez the vessel was disinfected, and on arrival at Southampton all persons on board were medically inspected, and their baggage disinfected before disembarkation. The troops and their families amounted to 1,560, and the crew numbered 183. In none of the persons on board was any illness found which could be suspected as plague. One of the passengers of the *Ionian* travelled on April 30th to Swansea. On the previous day, April 29th, he “fell out of sorts,” and continuing to feel ill called in a medical man at Swansea on May 2nd. The next day, May 3rd, the patient proceeded to Flint, and as he did not improve another medical man was summoned. The symptoms comprised high temperature, prostration, with dull and listless appearance, and slight crepitation at the base of both lungs. There was also some slight enlargement of the glands in the groin. These symptoms taken in conjunction with the history of the *Ionian's* voyage from Bombay, excited in the mind of the medical man a suspicion of plague; and he very properly took some material from the patient and sent it, with particulars of the case, to the Board for examination by their bacteriological expert. After careful application of bacterioscopic tests, Dr. Klein was able to state definitely that there was no evidence, whatever, pointing to plague.

No other suspected case of plague was reported to the Board during 1906 in any inland district of England and Wales.

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## APPENDIX A., No. 8.

The MANIFESTATIONS of CHOLERA throughout the WORLD  
during 1906; by DR. R. BRUCE LOW.

## INDIA.

The mortality from cholera in India during 1906 was the highest recorded since 1900. The number of deaths certified from the disease during the year was 713,664, as compared with 441,786 in 1905, and 193,657 in 1904. The Provinces which suffered most were Bengal, 192,596 deaths; the United Provinces of Agra and Oudh, 149,549 fatal cases; Madras, 142,811; and Eastern Bengal and Assam, 108,278.

The deaths from cholera, year by year, in India during the last 11 years are given in the subjoined table :—

India.	1896.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Cholera—Deaths	468,070	509,276	152,711	171,410	822,282	286,634	224,135	314,311	193,657	441,786	713,664

During the last 11 years no fewer than 4,404,936 deaths in India have been certified to be due to cholera, in addition to cases not reported or not recognised.

During the same period 4,664,902 lives have been lost from recognised plague, so that taking the period as a whole there has been no very marked difference between the ravages of the two diseases.

The facts, so far as they could be obtained, with respect to cholera in the various Provinces and Native States of India are given below :—

## BENGAL.

(Population, 1901, 49,891,164.)

The cholera deaths recorded in the Bengal Presidency during 1906 numbered 192,596, compared with 146,339 in the previous year. This was equal to a cholera death-rate of 3·81 per 1,000 of the population.

During the year under consideration no district entirely escaped the epidemic, and it is stated that no fewer than 21,365 villages were invaded. The largest numbers of deaths were certified in the months of April and May. The following table shows month

by month the number of cholera deaths reported in the Presidency during 1906 :—

Bengal.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths	10,263	9,537	13,795	23,836	23,623	17,108	17,288	15,022	13,505	18,964	14,344	15,231	192,596

Of the total cholera deaths 101,066 were males and 91,530 females. The highest number of deaths occurred in the following districts :—Purnea 17,579, Bhagalpur 17,190, Midnapore 13,406, Champaran 12,328, and Muzaffarpur 12,072. The total cholera deaths reported in the City of Calcutta during the year were 2,504.

The treatment of suspected wells by means of permanganate of potash was carried out in a number of places with good effect, but in some localities, owing to the hostility of the inhabitants, this measure had to be abandoned.\*

The subjoined table gives the number of cholera deaths recorded in the Bengal Presidency during each of the last 10 years, 1897 to 1906 inclusive :—

Bengal.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Cholera—Deaths	196,247	65,020	107,678	345,878	110,753	150,971	203,370	137,701	146,339	192,596

*Calcutta.*—The deaths recorded from cholera in Calcutta during 1906 numbered 2,504, as compared with 2,323 in the previous year. The subjoined table gives month by month the number of cholera deaths registered during 1906 :—

Calcutta.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths ..	235	316	170	349	209	143	51	46	72	137	334	442	2,504

Of the 2,504 deaths, 1,745 occurred among males and 759 among females. The deaths ascribed to diarrhoea and dysentery in Calcutta during the same period amounted to 2,734.

The increased number of cholera deaths reported in October, November and December is said to have been due to the large number of persons from Calcutta who attended the *Ras* festival in October. Many of these persons, it is said, developed cholera on their return. It is thought that bathing in the Ganges by the Hindus was one of the means by which cholera infection was spread.

\* Annual Report of the Sanitary Commissioner for Bengal for 1906, by Lieut. Colonel F. C. Clarkson, I.M.S.

The average annual number of cholera deaths in Calcutta for the 10 years period ended December 31st, 1905, was 2,101, so that the number for 1906 (2,504) was not greatly in excess of the annual average.

#### EASTERN BENGAL AND ASSAM.

(Population, 1901 Census, 29,812,735.)

Deaths from cholera in this Province during 1906 numbered 108,278, a death-rate of 3·63 per 1,000 of the population. These cholera deaths were distributed throughout the five divisions of the Province as follows :—Dacca division, 38,186 deaths ; Chittagong division, 18,393 deaths ; Rajshahi, 18,017 deaths ; Surma Valley division, 7,544 deaths ; and Assam Valley districts division, 26,138 deaths.

In the Dacca division the largest number of cholera deaths in 1906 were referred to the districts of Mymensingh (population, 3,915,068) 14,375 deaths, and Bakarganj (population, 2,291,752) 12,859 deaths. In the Assam Valley districts division the chief sufferer was the district of Kamrup (population, 589,187), which yielded 12,465 deaths from cholera.

The distribution of the cases throughout the months of 1906 is shown in the appended table :—

Eastern Bengal and Assam.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths ..	19,278	9,038	9,484	13,441	13,489	7,137	4,806	2,528	1,427	2,122	8,047	16,487	108,278

Of the 108,278 deaths, 55,884 were males and 52,394 females.

The cholera prevalence of 1906 was a continuation of the severe outbreak which occurred in the latter part of 1905, when in the months of October, November and December the cholera deaths were respectively 28,649, 39,982 and 41,710, a total of 110,341 deaths from cholera in the last three months of 1905.

The local manifestations of cholera in the various districts were associated with the use of contaminated water supplies.\* Where wells were suspected to be polluted they were treated with permanganate of potash, a process which the majority of Civil Surgeons in the Province regard as of great value in restraining the development of cholera outbreaks.

#### MADRAS PRESIDENCY.

(Population, 1901 Census, 42,397,522.)

The cholera epidemic of 1906 in this Presidency was the most severe which has been experienced since 1897. No fewer than 142,811 persons died from cholera in 1906, compared with 16,888

\* Annual Sanitary Report of the Province of Eastern Bengal and Assam for the year 1906, by Lieut.-Colonel E. C. Hare, I.M.S., Sanitary Commissioner.

in 1905. The heaviest mortality was experienced in the districts of Kistna, Godavari, Kurnool, Ganjam and Tinnevely. The progress of cholera in the Madras Presidency in 1906, month by month, is shown in the appended table :—

Madras Presidency.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths ..	2,774	1,247	836	1,015	3,153	14,531	29,968	36,496	30,477	12,954	8,690	10,690	142,811

The deaths were divided among the sexes as follows :—75,047 males and 67,764 females. The height of the epidemic was reached in July and August.

Considerable use was made of permanganate of potash for the purification of polluted sources of water supply, especially in invaded localities, and, it is said, with good results.\*

Only 540 out of the total 142,811 deaths from cholera were referred to the City of Madras.

The subjoined table shows the number of cholera deaths registered in the Madras Presidency during each of the last 10 years, 1897 to 1906, inclusive :—

Madras Presidency.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Cholera—Deaths ..	148,445	65,444	29,082	60,662	81,370	29,789	27,393	23,109	16,898	142,811

#### BOMBAY PRESIDENCY.

(Population, estimated to 1905, 25,468,209.)

Cholera increased considerably in this Presidency in 1906 as compared with the previous year. The Sanitary Commissioner for the Government of Bombay states, in his Annual Report for 1906, that 79,599 persons were attacked by cholera, and of these 46,119 died, a case mortality of 57·9 per cent. In 1905 the cholera deaths numbered only 5,396. The subjoined table gives the number of deaths from cholera month by month for 1906 in the Bombay Presidency :—

Bombay Presidency.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths ..	—	131	2,084	11,047	10,862	6,477	8,428	4,393	1,374	596	508	249	46,119

\* Annual Report for 1906 of the Sanitary Commissioner for the Madras Presidency.

Of the total 46,119 deaths 23,740 were males and 22,379 females. The districts (collectorates) in which the largest number of cholera cases were recorded were Poona with 11,242 attacks, 6,466 fatal; Satara 9,851 attacks, 5,431 fatal; and Nasik 8,127 cases, 4,354 fatal. As usual, the chief source of spread of the disease was polluted drinking water; wells and pools used as sources of supply were sometimes infected by pilgrims. The treatment of wells and pools by the permanganate of potash method was carried out as a protective measure, but in some districts it was found difficult of application owing to the strong prejudices of the villagers.

In addition to the 46,119 cholera deaths certified during the year, there were recorded 61,736 deaths from diarrhoea and dysentery.

The mortality from cholera in 1906 has only been exceeded twice during the last 20 years, viz., in 1897, when 57,109 deaths from the disease were registered, and 163,889 in 1900; both of these latter were years of famine.

*City of Bombay.*—The total cholera cases and deaths registered in the City of Bombay during 1906 were respectively 1,484 and 1,241 as compared with 29 and 26 in 1905. The disease persisted in Bombay throughout the year as may be seen by the subjoined table:—

City of Bombay.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Reported Cholera—													
Cases.. ..	1	1	26	187	118	130	423	313	176	31	47	31	1,484
Deaths .. ..	—	1	20	147	100	114	337	284	158	27	26	27	1,241

The case mortality was 83·7 per cent.

The outbreak was due to importation of infection by pilgrims returning from other districts in the Presidency, especially Poona. In the second quarter of the year 27 cholera-infected persons were discovered ill on their arrival in the City. The development of the disease was fostered by the deficient rainfall and other famine conditions.\*

#### PUNJAB.

(Population, 1901 Census, 24,533,088).

The deaths certified from cholera in the Punjab during 1906 numbered 4,232 as compared with 2,197 in 1905. In the early months of 1906 the Province was practically free from cholera. Several local outbreaks in April and May were associated with pilgrims who had returned from Hardwar. The subjoined table

\* Annual Report of the Executive Health Officer, Municipality of Bombay, for 1906.

shows the incidence of fatal cholera, month by month, in the Punjab during 1906 :—

The Punjab.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths ..	2	—	1	5	35	79	831	1,537	1,225	507	17	3	4,332

Of the 4,332 deaths, 2,493 were males and 1,739 females.

The district which suffered more than the others was Lahore, where 1,480 cholera deaths occurred during the year.

Among the general measures employed to prevent the spread of the disease may be mentioned the treatment of local wells by permanganate of potash.\*

#### THE UNITED PROVINCES OF AGRA AND OUDH.

(Population, 1901 Census, 47,691,782.)

Cholera caused 149,549 deaths in the United Provinces of Agra and Oudh during 1906, as compared with 121,790 deaths in 1905. The prevalence of the disease lasted during the whole year, but reached its height in May. The subjoined table shows, month by month, the deaths certified from cholera in the United Provinces during 1906 :—

United Provinces of Agra and Oudh.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths..	829	2,769	2,019	18,422	31,564	24,965	7,309	9,073	18,493	20,055	10,657	3,397	149,549

Of the 149,549 cholera deaths, 76,863 were males and 72,686 females.

The cholera death-rate per 1,000 of the population was 3·14, the corresponding rate in 1905 having been 2·55.

The largest number of cholera deaths were registered in the following districts :—Basti, 16,020; Gorakpur, 15,132; Gonda, 14,845; Kheri, 9,270; and Jhansi, 8,450. But all the districts in the United Provinces suffered more or less during 1906.†

\* Annual Report of the Sanitary Commissioner, Lieut.-Colonel C. J. Bamber I.M.S., on the Sanitary Administration of the Punjab for 1906.

† Annual Report of the Sanitary Commissioner of the United Provinces of Agra and Oudh for the year 1906.

The following table shows the number of cholera deaths reported in the United Provinces during the last 10 years, 1897 to 1906 inclusive :—

United Provinces of Agra and Oudh.	1897.	1898.	1899.	1900.	1901.	1902.	1903.	1904.	1905.	1906.
Cholera—Deaths ..	44,208	2,508	8,142	84,980	58,995	25,160	47,159	6,617	121,790	149,549

## BURMA.

(Population, 1901 Census, 10,490,624.)

The total deaths from cholera registered in Lower and Upper Burma during 1906 numbered 7,872 as compared with 5,347 in 1905. The districts which yielded most deaths from the disease were Thayetmyo (population 213,075) with 1,417 fatal cholera cases, and Henzada (population 433,813) with 1,100. Both these districts are situated in Lower Burma. The number of cholera deaths registered in the Province of Burma, month by month, during 1906 is given in the subjoined table :—

Burma.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths registered.	304	342	238	519	414	733	1,653	1,496	691	442	342	702	7,872

Of these deaths, 4,546 occurred in males and 3,326 in females.

Of the total number, 5,529 cholera deaths were referred to Lower Burma and 2,343 to Upper Burma. The Sanitary Commissioner,\* Colonel W. G. King, I.M.S., attributes the outbreaks of cholera in Burma largely to the use of specifically contaminated water, the opportunities for pollution of the rivers by filth being many and frequent. The deaths registered from diarrhoea and dysentery during the year were 8,377; and it is admitted that there is a possibility that a number of these deaths may have been due to cholera and erroneously certified as having been caused by diarrhoea or dysentery.

## CENTRAL PROVINCES.

(Population, 1901, 14,627,045.)

There were 38,768 deaths from cholera registered in the Central Provinces during 1906 as compared with 1,217 in 1905 and 2,967 in 1904. The epidemic of 1906 reached its height in August.

\* Report on the Sanitary Administration of Burma for the year 1906.

The course of the disease is shown in the table below, which gives the number of deaths certified in each month of 1906 :—

Central Provinces.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total in 1906.
Cholera—Deaths..	1	335	640	1,876	3,837	4,954	2,982	15,913	7,337	861	49	3	38,766

Cholera caused 1,496 deaths in the Sadar towns, which have a total population of 608,127.

#### OTHER INDIAN PROVINCES AND STATES.

In *Ajmer Merwara* there were 284 deaths from cholera in 1906. In the three preceding years there had been no deaths registered from this cause in the Province.

In *Rajputana* 4,714 deaths occurred from cholera. The Province had been practically free from the malady during the two previous years, only three deaths having been recorded from cholera in 1905 and one in 1904.

In *Coorg* only 10 deaths from cholera were certified during the year. There had been no deaths from the disease in Coorg during the four preceding years.

In *Central India* the cholera deaths numbered 10,147 during 1906, compared with only 27 in 1905 and 150 in 1904.

In the Native State of *Mysore* 7,223 persons lost their lives in 1906 from cholera. In the *Hyderabad Cantonment Stations* 1,061 deaths from this cause were certified as against 64 in the previous year. No deaths from cholera were reported in the Hyderabad Cantonments during 1902, 1903 or 1904.

#### CEYLON.

(Population, 1901 census, 3,565,954.)

Cholera appeared in Ceylon during the autumn months of 1906, invading most of the provinces, and attacking some 756 persons, of whom 449 died, a case mortality of 59.3 per cent. The main epidemic, however, was in the province of Uva, when 549 cases and 355 deaths occurred.

The subjoined table shows the incidence of cholera in Ceylon during the last five years 1902 to 1906 inclusive.

Ceylon.	1902.	1903.	1904.	1905.	1906.
Cholera attacks ...	179	46	7	—	756
Cholera deaths ...	116	23	4	—	449

## SIAM.

There was an outbreak of cholera at Bangkok in February, lasting till the end of April, during which period 224 persons were attacked and 198 died of the disease. The outbreak was associated with very hot weather and drought. A second but smaller outbreak of cholera occurred in Bangkok in August, when 17 cases and 12 deaths were reported.

A single case occurred on board the s.s. *Lena* while lying in Bangkok harbour in March. The ship sailed for England three days after the death of the patient, whose bedding and effects were thrown overboard at Bangkok. There were no further cases, and on reaching Gravesend on May 6th, all on board the *Lena* were found in good health.

## FRENCH EAST INDIAN POSSESSIONS.

Cholera prevailed in Chandernagor, Pondicherry and Karikal during July, August and September, some 1,708 deaths being certified from this cause. The chief sufferer from the disease was Chandernagor. In Cochin China a few cases were reported. At Laos, in the province of Xien-Khong, in the region of Tham-Loi, the disease showed itself. Some cases were also observed in Hanoi, Bac-Ninh, and in Haiphong (Tonkin). Details of these outbreaks have, however, not been obtainable.

## STRAITS SETTLEMENTS.

*Singapore.*—During 1906 there were notified in Singapore 185 cases of cholera, 167 of which proved fatal. The bulk of the cases occurred in the months of April and May, no fewer than 134 of the attacks and 120 of the deaths being referred to that period.

*Province Wellesley.*—There was an outbreak of cholera in the Nibong Tebal district, 20 miles from Penang, during April and May; from the 10th of April to the 12th of May some 107 cases and 88 deaths were certified from the disease. Only two cholera cases were reported from Penang.

## FEDERATED MALAY STATES.

(Population, 1906, 915,000.)

*State of Selangor.* (Population, 1906, 283,619).—Only seven cases of cholera, four fatal, occurred in this state, at Port Sweetenham. All of them were Indian immigrants who had arrived from India in ships on board which cholera cases had occurred during the voyage.

*State of Perak.* (Population, 1906, 399,393).—Cholera broke out in the Krian district in the month of May. There were 66 cases and 32 deaths during the epidemic, which lasted about a fortnight. The origin of the outbreak is not stated.

## THE PHILIPPINE ISLANDS.

The cholera prevalence of 1905 was continued in the Philippine Islands during 1906, for the total reported cases during the year amounted to 10,186, and the deaths to 7,706, compared with 1,897 cases and 1,418 deaths during 1905.

*Manila.*—Of the above total for the Philippine Islands, 848 cases and 744 deaths were referred in 1906 to Manila, as against 255 attacks and 226 deaths in 1905. This epidemic is said to have been due to the specific contamination of drinking water. Some use was made of the anti-cholera prophylactic injections, but this protective inoculation was much discredited by an unfortunate occurrence in the Manila prison, where it had been employed with a view to protect the prisoners. Of 24 of the prisoners in the Bilibid jail who received the injections 13 died of plague. It is said that cultures of plague had got accidentally mixed up with the anti-cholera prophylactic fluid in the laboratory.

The cholera outbreak in Manila took on epidemic proportions towards the end of June, and continued with considerable severity during July, August, and September.

In the *Provinces* the epidemic reached its height during the third quarter of the year, more than half of the cases and deaths being referred to the months of July, August, and September. A number of provincial towns were invaded by cholera almost simultaneously in June, and this was attributed by the authorities to the specific contamination of the river Pasig, due to the copious use of human excrement by Chinese to manure their gardens on the banks of the river.\* The provinces which suffered most were Bulacan, Iloilo, Pampanga and Rizal.

The manifestations of cholera in the Philippine Islands since 1902 are shown in the appended table :—

Philippine Islands.	1902.	1903.	1904.	1905.	1906.
Reported cholera attacks ...	125,560	40,465	127	1,897	10,186
Reported cholera deaths ...	80,568	28,746	85	1,418	7,706
Case mortality ...	64.2	71.1	66.9	74.7	75.6

## CHINA.

*Shanghai.* (Chinese population, 475,000.)—An outbreak of cholera occurred in Shanghai in September and October, 1906. Suspicion that cholera had appeared was first excited by the

\* The Philippine Commission passed an Act, in 1906, making it a penal offence to use human excrement for manuring gardens. Two important sections are as follows :—(1) No farmer, market gardener, or other person or persons shall use any human excreta, excrement, dejects or the contents of any water or earth closet, privy vault, cesspool, latrine, pail or other receptacle for human faeces or urine as a fertiliser for any land on which is grown any article or product intended for human food or human consumption, or allow any human excrement, excreta, or dejects to be sprinkled or applied in any manner, or for any purpose to any crop, product, or vegetable growing on said land.

(2) A fine of not less than 25 dollars, nor more than 100 dollars, or imprisonment not to exceed six months, or both, shall be imposed upon each guilty person. (U.S.A. Public Health Reports, October 5th, 1906.)

excessive number of deaths from diarrhoea among the native population, and investigations made by Dr. Stanley, the Medical Officer of Health, confirmed at once the suspicion. Altogether 197 deaths were certified from cholera, but this does not include the many cases which were first regarded as fatal diarrhoea. The incidence of the disease was chiefly in the Yangtsepoo and Hongkew districts. Only four foreigners died of the disease, one of them on board the German warship *Lachs*, which was lying in the port.\*

*Hong Kong.* (Population, 1906, 326,961.)—Only two fatal cholera cases were reported from Hong Kong during 1906, both of them Chinese dock labourers, one occurring in May and the other in July.

*Foochow.* (Population, 1906, 650,000.)—During September a few cases of cholera came under observation in Foochow, but there was no epidemic.

*Hankau or Hankow.* (Population, about 600,000).—This town is situated at the confluence of the Yangtse-kiang and the Han-kiang, about 600 miles from the mouth of the former river. There were reported in Hankau during the first week of October, 1906, some 24 deaths from cholera, but no details of the outbreak beyond the mention of these deaths have been made public.

#### JAPAN.

There was no epidemic of cholera in Japan during 1906, but two imported cases were reported from the port of Moji during October. These had been landed from a vessel which had arrived from Shanghai, where about that time an outbreak had occurred.

#### FIJI ISLANDS.

A report appeared in the London papers of May 11th, 1906, stating that on board the s.s. *Faukla*, carrying coolies from India to Suva (Viti Levu), the capital of the Fiji Islands, cholera had broken out, attacking 124 of the coolies, of whom 61 had died. But this report, so far at least as can be ascertained, has not been officially confirmed, and it is not clear at what point of the voyage between India and Suva the outbreak took place. Presumably the infection was contracted at an Indian port.

#### RUSSIA.

It may be remembered that during 1905 cholera appeared in Russian Poland in the basin of the Vistula, and that towards the end of that year the disease was dying out. The entire cessation of the epidemic was announced officially on February 6th, 1906. But during January, 1906, some 102 cases with 60 deaths were notified, viz., one fatal case at Warsaw, 29 cases and 11 deaths in the government of Lomza, and 72 cases, 60 fatal, in the government of Plock.

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\* Annual Report of the Medical Officer of Health for Shanghai, for the year 1906.

According to an official report,\* published at Warsaw, the outbreak of cholera in Russian Poland from September, 1905, to February, 1906, comprised 445 notified cases and 220 certified deaths.

From February, 1906, till July, 1907, nothing further was heard of cholera in Russia. But in the last-named month a serious outbreak occurred in various governments situated on the River Volga, and spread thence over a large part of European Russia. Details of this more serious epidemic are reserved for the Annual Report for 1907.

### THE UNITED KINGDOM.

No cases of cholera were notified in Great Britain and Ireland during 1906, although three ships technically infected by the disease arrived in English ports during the year. In these instances the illness had occurred in the port of departure before the vessel sailed from the Far East, or had occurred on the voyage a few days after leaving the port of departure.

Some alarm, however, was created in October by the arrival at Queenstown of the s.s. *Peruviana*, which had come from Rotterdam *via* Penarth. A rumour had been in circulation early in October that two cases of cholera had occurred in Rotterdam, but no confirmation of this rumour was forthcoming. But as the *Peruviana* had come from Rotterdam, and as on arrival two of the crew were dead and four others seriously ill with gastro-intestinal symptoms, there appeared to be some grounds for the alarm that arose. Post-mortem examinations were made, followed by bacterioscopic investigation carried out by experts employed by the Irish Local Government Board. No evidence, however, was found to support a diagnosis of Asiatic cholera. It appeared on further inquiry that the vessel had called at Penarth on the voyage from Rotterdam, and that while in that port several members of the crew had partaken of mussels somewhat freely. These mussels it is said had been collected from piles, covered with copper, which supported a wooden pier. Of the two sailors who died, one was a Russian, the other a German.

### EGYPT.

No cholera was reported in Egypt during 1906, but six ships technically infected arrived at Suez from the East having had cases of cholera on board in the port of departure or during the voyage. In four of the instances single fatal cases had occurred on board and the patients had been buried at sea; in one instance four cholera cases developed on board while the ship was in the port of Basein (Burma) and in the remaining instance a patient who had fallen ill on the ship while in the port of Rangoon was landed at the port hospital there. In each instance the ship on arrival at Suez was found after medical inspection to be free from infection and was allowed to pass through the Canal.

\* For this and other information I am indebted to Dr. J. Polak, Medical Officer of Health for the City of Warsaw.

## APPENDIX B.

## No. 1.

REPORT on FURTHER RESEARCH in connexion with PLAGUE;  
by Dr. KLEIN, F.R.S.

## A.—THE ORGAN PROPHYLACTIC OF PLAGUE.

In last year's Report I have shown that by injection of the dried material of necrotic organs of a guinea-pig (the subject of induced sub-acute plague) white rats are furnished with pronounced immunity against subsequent infection with a fatal dose of *B. pestis*. The experiments in question further demonstrated that not only the watery extract of the organ prophylactic possesses the above immunising potency, but also that the clear filtrate of this watery extract is similarly efficacious if employed in a slightly larger dose. By heating this filtrate to 70° C. for ten minutes one of the objections that may be urged against the use of the organ prophylactic—viz., possibility of not being reliably devoid of living bacilli or cocci—could, it was pointed out, be obviated. This sterilised filtrate, which is a clear light brown fluid, may moreover be easily and permanently preserved in sealed tubes, and thus rendered available for distribution in this and other countries.

As an outcome of the experimental work referred to I have in my possession a bottle containing about 48–50 grammes of the dried necrotic organs (bubo, liver, spleen, and lung) of about 12 full-sized guinea-pigs which had succumbed to sub-acute plague—*i.e.*, which had died between the fifth and tenth days after cutaneous inoculation. This material, as was described in my report, had been dried at 46° C. for three days, then finely powdered and dried additional three days at 37° C., and finally permanently preserved in a closed sterile glass bottle. Further, this material had been standardised, with the result that it was found that 15.6–20 milligrams used as watery emulsion of the raw material, and 25–30 milligrams used as heated filtrate, constitute reliable doses for protecting a white rat of 120–200 gram body weight against cutaneous inoculation with virulent *B. pestis*.

The material in question preserved in a sterile glass bottle and stored in a cool dark place has in no way deteriorated its efficacy. For more than 18 months I have used it with complete success in many further experiments, and have further ascertained that the above stock, while capable of acting as a reliable prophylactic, is devoid of lethal toxic effect in the rat such as was observed in some of my earlier experiments.

The experiments conducted in the present year with the filtrate sterilized by heat were designed for the purpose of ascertaining any difference in value of the filtrate prepared from the watery emulsion and that prepared from the salt solution emulsion

of the raw material; as also what is the optimum temperature in the heating of the filtrate after sealing it up in glass phials for rendering it sterile while at the same time retaining its potency.

*Experiment 26.\**—This experiment is a preliminary experiment to test the efficacy of the preserved filtrate.

On June 13th, 1906, five adult white rats. Each rat received subcutaneously one cubic centimetre (representing 50 milligrams of raw organ material) of clear filtrate of watery emulsion. This filtrate, prepared in the fashion described in last year's report and heated on two occasions to 70° C. for 10 minutes, had been preserved in a sealed phial for 5½ months.

In preparation of the filtrate in question the powdered dry material is weighed out, thoroughly rubbed up in sterile mortar with the required amount of sterile distilled water or of sterile salt solution, passed through fine filter paper in a sterile glass filter, and finally received in a sterile test tube. The test tube, which is of the long thin kind, and contains 5 cc. of filtrate, occupying half of the total space of the tube, is then plugged with sterile wool. The filtrates at this stage are clear slightly brown fluids. After heating the tube to 70° C. for 10 minutes, the contained fluid will be observed to become faintly opalescent. The tube is now sealed by heating and drawing out the glass midway between the fluid and the cotton wool plug, after which it should be put by in a cool, dark place. In the course of a few days it will be noticed that the fluid has again become quite clear, and that it has deposited a small amount—often a mere trace—of a fine powder precipitate.

Twelve days after the injection of the filtrate—that is on June 25th—the five rats were cutaneously inoculated with a trace of 24 hours' agar culture of *B. pestis* derived from the bubo of a monkey that had died of acute (inoculated) plague. At the same time one control rat was cutaneously inoculated with a like amount of the same culture of *B. pestis*.

The control rat was distinctly ill on June 26th, and was found dead in the morning of June 27th. Post-mortem examination showed hæmorrhagic inguinal bubo packed with *B. pestis*; the spleen enlarged, dark, firm, crowded with *B. pestis*; the liver congested and crowded with *B. pestis*; both lungs deeply congested, and with petechiæ, full of *B. pestis*; heart blood contained numerous *B. pestis*. As the rat had died within 46 hours or less, and as it showed the typical appearances of acute plague, it must be concluded that the culture—a mere trace having been used—was of high degree of virulence.

The five prepared rats inoculated with the same *B. pestis* culture showed at no time subsequently any illness; they remained unaffected, and all survived.

It is shown, therefore, that the twice heated watery filtrate sealed up and preserved for 5½ months possessed full protective potency. The experiment at the same time proves that the amount of filtrate, 1 cc., prepared from as much as 50 milligrams of raw material was devoid of ability to produce acute toxic effect.

*Experiment 27.*—On July 5th three half-grown rats injected with clear newly prepared filtrate of the stock organ prophylactic,

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\* The numbers here given refer to numbers in the laboratory note book of series of experiments made with the stock prophylactic during the year.

each receiving subcutaneously an amount (1 cc.) corresponding to 25 milligrams of raw powder.

A portion of the same filtrate was heated to 65° C. for 10 minutes and injected in same dose as above into three other half-grown rats.

All the animals remained unaffected.

On July 21st these six rats, together with a full-grown control rat, were each inoculated cutaneously with a trace of a 24 hours' agar culture of *B. pestis* of same stock as used in Experiment 26 (monkey bubo).

The control rat was found dead in the evening of July 23rd, and post-mortem examination showed acute typical plague with abundance of *B. pestis*, as in the previous experiment.

The six protected rats remained unaffected and survived.

This experiment shows that the filtrate whether unheated or heated to 65° C. for ten minutes possessed full protective efficacy in doses corresponding to 25 milligrams.

*Experiment 28.*—Four small rats (60–80 grammes in weight) were injected subcutaneously on September 19th with filtrate of watery emulsion of organ prophylactic. The crude material was a mixture of the organs of guinea-pigs, rats, and mice that had died of acute plague, which organs had after mincing been dried at the temperature of the room. The dried powder had been kept in stoppered bottle for over a year. The filtrate prepared from this powder was heated to 65° C. for ten minutes. Each rat received an amount of filtrate corresponding to 30–37 milligrams of the raw material.

One of these rats was found dead on September 22nd, a second one on September 27th. In both instances there were signs of congestion at the seat of injection, the spleen was enlarged, the lungs congested. No bacteria of any kind could be detected in these tissues either in films or by culture.

The remaining two rats were, together with a control rat, inoculated cutaneously with virulent *B. pestis* (monkey bubo, 24 hours' agar culture) on November 1st.

The control rat was dead on November 3rd, with acute typical plague. The two protected rats remained unaffected.

This experiment shows that even though the material contains appreciable amounts of toxin (lethal to small rats) heating its watery filtrate to 65° C. for 10 minutes does not interfere with its toxic potency nor with its power to protect against virulent plague.

*Experiment 29.*—This experiment was made on September 21st. Two sets of rats (three in each instance) were used : (a) one set of three rats was injected subcutaneously with clear filtrate prepared from an emulsion in physiological salt solution of the stock organ prophylactic (necrotic organs of guinea-pigs, dried at 46° C.); (b) the other set of three rats was injected with clear filtrate prepared from an emulsion in sterile distilled water of the same stock prophylactic. In each set each rat received an amount of filtrate corresponding to 25 milligrams of dried material, and with results as follows :—

(a) set : one rat was found dead on September 22nd, a second rat on September 24th, and the third rat on September

26th. In all these three cases the appearances were those of toxin poisoning, no bacteria were found in the spleen or at the seat of injection.

(b) set : all three rats remained unaffected.

After a fortnight the rats of (b) set were, together with a control rat, cutaneously inoculated with virulent *B. pestis* (24 hours' agar culture of monkey bubo). The control rat died of acute typical plague in 60 hours, whereas the three protected rats remained wholly unaffected.

This experiment indicates that the organ prophylactic contains toxic substances readily extractable by salt solution and acting lethally in a dose of 25 milligrams ; and that on the other hand the watery extract does not contain similar toxins, at any rate not in lethal or pathogenic amounts. From physiological chemistry it is known that salt solution readily extracts and dissolves bodies belonging to the globulin group, and it is to be inferred therefore as probable that some of the toxic bodies of the dried plague organs belong to this group ; and further it would seem to follow that for protective purposes and to avoid toxic accident the organ prophylactic filtrate should be prepared and the filtrate used as a watery extract.

*Experiment 30.*—This experiment, instituted September 25th, is in a certain sense a repetition of the preceding one, except that the filtrates used, both the one prepared with salt solution and the other with water, were heated to 65° C. for 10 minutes. The dose employed was 25 milligrams for each of three rats in set (a) of filtrate of salt solution extract of stock organ prophylactic, and for each of three rats in set (b) a corresponding amount of filtrate of watery extract of this prophylactic.

As a result, all three rats of set (a) died of toxin poisoning (one after 9 days, the other two after 10 days), whereas all three rats of set (b) remained unaffected.

These set (b) rats, as also a control rat, were a fortnight later tested with virulent *B. pestis*. The control rat died in about 50 hours, but the three protected rats remained alive.

The inference as to the salt extract containing toxic bodies probably of the globulin series is further confirmed by the following observation. As already mentioned, the filtrates, both those prepared from the watery emulsion and the salt emulsion, are clear light brown fluids. But on heating them at 65° C. a marked difference is observed. The watery filtrate becomes very faintly opalescent, whereas the salt filtrate turns markedly opalescent, and after standing deposits a relatively large amount of powdery precipitate at the bottom of the tube. This is altogether consistent with a view that the salt filtrate contains a marked amount of globulins (all coagulable by heat of 60° to 65° C.).

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Several further experiments, experiments 31, 32, 33, and 34, were made with samples of watery filtrate of the stock organ prophylactic which had been heated to 60° C., 62° C., and 65° C. for 10 minutes in the autumn of 1906, and then sealed in glass tubes as previously described.

They all proved fully protective for half-grown to adult rats in doses of 25 to 30\* milligrams when used at the present date, March 1907. It is not necessary to detail these other experiments since they are of precisely the same character and yielded the same result as those described in experiments 27, 29, and 30. All these stock filtrates, whether heated at 60° C. or at 65° C., proved on culture quite sterile. Nevertheless in order to fully secure the destruction of any cocci or bacilli accidentally present, and thus insure the filtrate remaining sterile in the sealed tubes, it is perhaps the safer plan to heat all such filtrates to 65° C. for 10 minutes, since this heat while completely sterilising the fluid may be trusted to leave its prophylactic potency quite unimpaired.

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#### B.—THERAPEUTIC USE OF ORGAN-EXTRACTS OF ANIMALS RECOVERED FROM PLAGUE.

In the report of the Medical Officer, 1901-1902 (pp. 360 and *passim*), a number of observations are recorded by me which show that the blood of rodents (guinea-pigs, rabbits, and rats) which had been repeatedly injected with plague, and which had recovered from the disease, does not contain appreciable amounts of germicidal substances; of substances that is capable of neutralizing the action of the *B. pestis* when introduced along with the latter into the bodies of unprepared animals.

In this sense, as I point out in the report in question, plague differs widely from some other infectious diseases, *e.g.*, cholera, diphtheria, &c., in regard of which germicidal substance or substances have been demonstrated to exist in the blood of the immunised animal. But since it cannot be doubted that the body of the plague immunised animal does, as a matter of fact, include germicidal materials, it follows that these latter have to be sought for otherwise than in the blood of the animal. And since it has been shown in last year's report that the organs of a plague animal (lymph glands, spleen, liver) contain plague toxin, it is justifiable to assume that in these organs also antitoxins and germicidal substances will probably be found in the immunised animal. If this should prove the case it is obvious that extracts of these organs, taken from an animal which has become immunised against plague, might have the power to act therapeutically under certain conditions; that is to say, extract of the organs of an immunised animal might, when injected into other plague-infected but not immunised animals, neutralise or cut short the disease.

This presumption has been put to the test in a large number of experiments begun by me in 1905 and continued during the last two years. Dr. Mallannah, too (Bacteriologist to H.H. the Nizam of Hyderabad), has published in the "Lancet," January 26th, 1907, a number of interesting experiments on precisely similar lines. This observer shows that using for injection 100, 120, 150, or even

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\* In one series (experiment 31) 20 milligrams proved quite sufficient for small rats of body weight of 60-80 grammes.

200 to 250 milligrams of the dried organs of immunised rabbits positive therapeutic effect (*i.e.*, recovery) can be secured in a large percentage (50-75) of the infected animals (guinea-pigs and rats, brown and white). He prepared and used the organ-material in precisely the same manner as that described by me in last year's report, injecting the watery emulsion half-an-hour or 24 hours after infection of an animal with virulent *B. pestis*.

I proceed now to describe my own experiments on this subject.

#### SERIES I.

*Experiment 1.*—The laboratory stock of the dried powder of the necrotic organs of guinea-pigs dead of sub-acute plague, which, when used as filtrate of watery emulsion corresponding to 25 milligram doses, had acted reliably as prophylactic for half-grown and adult rats was tested in the first instance as to any therapeutic value that it might happen to possess.

Four rats were cutaneously inoculated on April 24th from a 24 hours' agar culture of monkey bubo.\* Three of these rats were next day (April 25th) subcutaneously injected with the filtrate (25 milligrams each) of the above stock prophylactic, the fourth rat being kept in control.

On April 26th the three test rats received subcutaneously a further and like dose of the filtrate of the prophylactic. At this time all the four rats (three treated and one control) were distinctly quiet and not feeding.

On April 28th all four rats were found dead. On post-mortem examination they showed typical acute plague: inguinal bubo and typical plague spleen crowded with the *B. pestis*.

It follows therefore that the stock prophylactic exerted no therapeutic action on rats, when injected 24 and 48 hours after infection of these animals with plague.

*Experiment 2.*—A monkey had been injected subcutaneously on February 24th with turbid emulsion of a subculture derived from the spleen of a human fatal case of plague. This monkey had been found dead on March 2nd with typical acute plague: bubo and liver crowded with *B. pestis*, spleen enlarged and contained numerous *B. pestis*. The liver and the bubo (minced) had been dried at 46-47° C., powdered, again dried, and preserved for prophylactic experiments. Further, this material had been used as watery emulsion in doses of 15.6 milligrams, and as filtrate of the watery emulsion in doses of 25 milligrams, for prophylactic purposes on half to full grown rats, and had been found protective of these animals.

On April 18th four rats and one control rat were cutaneously inoculated from a 24 hours' agar culture of monkey bubo. Thirty minutes later the four rats (not the control) each received subcutaneously 25 milligrams of the above monkey liver stock. On April 19th, *i.e.*, 24 hours after infection, the four rats were re-injected

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\* In all instances in this and in subsequent experiments the cutaneous inoculation with *B. pestis* was performed on the left side of the body, the subcutaneous injection of the therapeutic in the right groin. All rats referred to were tame rats half-grown or at least not fully adult.

each with 25 milligrams of watery emulsion of same monkey liver stock. By this time the four rats, as also the control rat, were quiet and not feeding.

On April 21st the control rat and one of the treated rats were found dead, and on post-mortem examination both animals showed acute typical plague—*i.e.*, crowds of *B. pestis* in the bubo and the spleen. On April 23rd a second treated rat was found dead; very few bacilli could, however, be discovered in its bubo or in its spleen.

The other two rats recovered and survived.

*Experiment 3.*—Seven rats were inoculated cutaneously on May 10th with a 24 hours' agar culture of the spleen of a rat dead of acute plague in 36 hours.

(a) Half-an-hour afterwards three of the above rats were injected subcutaneously, each with 50 milligrams of the filtrate of the dried liver powder (watery emulsion) of the monkey as used in previous experiment.

(b) After 24 hours, *i.e.*, May 11th, three other rats of above lot were subcutaneously injected each with 50 milligrams of the filtrate of monkey liver. The seventh rat served as control. The result of this experiment was as follows:—

On May 12th, *i.e.*, within 44 hours, the control rat and two rats of lot (b) were found dead of typical acute plague: their bubos and spleens crowded with *B. pestis*. On May 14th (Sunday, May 13th, intervening) all the other rats (three of lot (a) and that remaining of lot (b)) were found dead of typical acute plague.

From these experiments it appears that two injections (experiment 2) of the monkey liver prophylactic in 25 milligram doses did exert a therapeutic effect in a certain percentage of rats (50 per cent.), whereas a single injection of 50 milligram doses had no appreciable therapeutic effect, whether administered half-an-hour or 24 hours after plague infection.

*Experiment 4.*—On May 14th five rats were inoculated cutaneously with lung juice of a rat dead the same morning (May 14th) from acute typical plague, the juice of the animal's inflamed lung being crowded with *B. pestis*.

On May 15th four of these rats received subcutaneously 40-50 milligrams each of heated (70° C.) filtrate of watery emulsion of the monkey liver used in previous experiment. The fifth rat served as control. This control rat was found dead in the morning of May 19th from acute typical plague; but the four treated rats were then lively and well, and they remained so.

*Experiment 5.*—On May 25th five rats were inoculated cutaneously with a 24 hours' agar culture of monkey bubo.

On May 26th four of these rats were injected subcutaneously with 50 milligrams each of the heated (70° C.) filtrate of the monkey liver. The fifth rat served as control.

All five rats were found dead in the afternoon of May 28th.

Thus, here are two experiments performed in the same manner but differing only in the circumstance that material for the preliminary infection was from different stocks of *B. pestis*. The therapeutic administered was, for instance, of the same kind

(heated filtrate of monkey liver) in the same dose (50 milligrams per rat), the lapse of time (24 hours after infection with *B. pestis*) was the same, and the rats belonged all to the same brood. On the other hand, the infecting material was different. In experiment 4 it was lung juice of a plague dead rat; in experiment 5 a 24 hours' agar culture of virulent monkey bubo stock. That these two plague stocks differed in virulence is certain. Thus the infecting material in experiment 5 (24 hours' agar culture of monkey bubo) was distinctly of a more noxious character than that of experiment 4 derived directly from the lung juice of a rat. The control rat of experiment 4 lived five days after infection, whereas in experiment 5 the control rat lived three days only. This experience is quite in accordance with other observations which I have repeatedly made, viz., that a 24 hour old agar culture of *B. pestis* proves of distinctly greater virulence than plague material directly taken from a plague animal.

It would seem that this difference in the virulence of the infecting material may be the cause of the different results recorded above, inasmuch as in experiment 4 the effect of the infection being slower (the disease lasting longer) there was afforded better opportunity for the action of the therapeutic than in experiment 5, in which, owing to the higher virulence of the infecting material, the effect of the infection was quicker, and the induced disease to a corresponding extent more quickly fatal.

*Experiment 6.*—In this experiment guinea-pigs were substituted for rats in making test of the therapeutic value of the prophylactic.

On June 19th five guinea-pigs were cutaneously inoculated with a 24 hours' agar culture of monkey bubo. Next day all the animals seemed unaffected. On June 21st they were all quiet, slightly off their feed, and showed small bubos. Four of these animals were now subcutaneously injected each with 50 milligrams of heated filtrate of watery emulsion of monkey liver stock. The fifth guinea-pig served as control.

On June 22nd the four treated guinea-pigs were re-injected, each with 62 milligrams of the same material, i.e., of heated watery filtrate of monkey liver stock.

On June 23rd one of the treated guinea-pigs was found dead: early sub-acute plague, crowds of *B. pestis* in bubo, spleen, and liver.

On June 25th the remaining three treated and the one control guinea-pig were found dead: sub-acute plague in all. Result, therefore, was nil therapeutically.

*Experiment 6a.*—On September 18th four guinea-pigs were inoculated cutaneously with a 24 hours' agar culture of monkey bubo stock.

On September 19th three of these animals received each 62.5 milligrams of the guinea-pig organ prophylactic. The fourth guinea-pig was kept as control.

On September 24th the control and two of the treated guinea-pigs were found dead, all showing typical sub-acute plague. The third treated guinea-pig was found dead on September 28th with

extensive sub-acute plague: necrotic bubo, necrotic nodules of spleen, liver, and lungs, all tissues prevailed by *B. pestis*.

The result, therefore, was again nil; neither the monkey liver prophylactic nor the guinea-pig organ prophylactic manifesting any therapeutic action on the plague-infected guinea-pigs.

## SERIES II.

In this series the dried powder of the liver and spleen of a monkey was used as the therapeutic agent. This monkey had recovered from plague infection succeeding prophylactic infection. Its history is as follows:—

The monkey had been injected subcutaneously on May 14th with 25 milligrams of twice heated (watery) filtrate of the guinea-pig organ prophylactic. A fortnight later (May 28th) this animal received subcutaneously in the inguinal region 10 divisions of a Pravaz syringe ( $\frac{3}{4}$  of a cc.) of a turbid emulsion of a recent agar culture of virulent *B. pestis* (monkey bubo stock). The animal remained, however, unaffected. It was killed on June 27th, that is about a month after injection with plague material. Post-mortem: all the viscera were found normal, no enlarged glands to be noticed in the inguinal region. The liver and spleen were now cut out, minced and dried in the usual way (see former reports) for three days at 46° C., then powdered and finally kept for three days at 37° C., and preserved afterwards in stoppered bottle.

Two preliminary experiments were made with this material.

*Experiment 7.*—Four guinea-pigs were inoculated cutaneously on July 7th with a 24 hours' agar culture of monkey bubo stock.

On July 9th each animal received subcutaneously in watery emulsion 50 milligrams of the liver and spleen of the particular monkey killed on June 27th. On July 11th developing bubo was noted on the inoculated side in each animal, and all were quiet. Two were found dead on July 13th, the other two on July 16th. All showed typical sub-acute plague.

*Experiment 8.*—On October 3rd five guinea-pigs were cutaneously inoculated with fluid of the inguinal bubo of a guinea-pig dead of plague, this fluid being crowded with *B. pestis*. One hour after inoculation each of four was injected subcutaneously (in opposite side) with 100 milligrams of salt emulsion of filtrate of monkey liver. The fifth was kept as control. On October 5th (*i.e.*, 48 hours after plague infection) each of the four treated animals was reinjected each with 100 milligrams of the same salt filtrate. The animals seemed quiet, but had each distinct bubo on the inoculated side.

On October 6th the four treated guinea-pigs, as also the control, were found dead with typical acute plague, intestine showing numerous petechiæ, bubo, and spleen crowded with *B. pestis*.

This experiment shows that the infective material was exceptionally virulent, and that under these conditions a double

injection—each time of 100 milligrams—of the therapeutic agent under test had no effect whatever in modifying the course of the induced disease.

I did not deem it advisable further therapeutically to test this material since the animal from which it had been derived had not exhibited any marked plague illness by which its organs might presumably have become endowed with appreciably active anti-bodies.

### SERIES III.

In this series employment was adopted of the organs of rats which having been first "protected" by the plague prophylactic were afterwards infected cutaneously with virulent *B. pestis*. All have survived.

The history of this material is as follows :—

- (a) Four rats received on June 11th, subcutaneously, 20 milligrams each of the twice heated watery filtrate of the guinea-pig organ stock prophylactic. On June 21st they, together with a control rat, were inoculated cutaneously with a 24 hours' agar culture of virulent *B. pestis* (monkey bubo). The control rat was found dead on June 23rd with typical acute plague; whereas the "protected" rats remained unaffected and survived.
- (b) Two rats each received, subcutaneously, on June 20th, 20 milligrams of watery filtrate of the prophylactic as in the case of (a). On July 2nd these two rats, together with a control rat, were inoculated cutaneously with a 24 hours' agar culture of virulent *B. pestis* (monkey bubo). The control rat was found dead on July 4th with typical acute plague, whereas the "protected" rats remained unaffected and survived.

On August 1st all the above six treated rats being quite normal were killed. Post-mortem, all their organs appeared quite normal, no enlarged glands anywhere. Their livers and spleens were cut out, minced, and dried at 46° C. After completion of drying (three days at 46° C.) the material was powdered, placed in sterile bottle, and then kept for further three days at 37° C.; that is to say, the material was subjected to precisely the same treatment as was described in my last year's report on the organ prophylactic.

*Experiment 9.*—Four rats were inoculated cutaneously on September 19th with a 24 hours' agar culture of monkey bubo stock.

Three of these rats received subcutaneously, in watery emulsion, on September 20th, each 50 milligrams of the material prepared from the organs of the (a) and (b) rats referred to. The fourth rat served as control.

The control rat died of typical acute plague on the evening of September 21st—its spleen crowded with *B. pestis*.

One of the treated rats was found dead on the morning of September 22nd with typical acute plague; its spleen was crowded with *B. pestis*.

A second treated rat was found dead on the morning of September 24th, and the last of the treated rats was found dead on the morning of September 26th.

But in neither of these two last dying rats could any *B. pestis* be discovered, either by film specimens or by culture.

*Experiment 10.*—Four rats were inoculated cutaneously on September 25th with a 24 hours' agar culture of monkey bubo stock.

On September 26th three of these rats received each subcutaneously 50 milligrams of salt filtrate of the (a) and (b) rat therapeutic. The fourth rat served as control.

On September 27th the three treated rats were injected with 60 milligrams of same salt filtrate. On this day all the three treated rats, as also the control animal, seemed ill; they were quiet and not feeding.

On September 28th all four rats were dead or dying, two being found dead in the morning, while two died in the afternoon. Post-mortem of all four rats showed acute typical plague with abundance of *B. pestis* in the hæmorrhagic bubo as also in the spleen.

*Experiment 11.*—Four guinea-pigs were inoculated cutaneously on September 25th each with a 24 hours' agar culture of monkey bubo stock.

On September 26th three of these infected guinea-pigs were injected subcutaneously each with 50 milligrams of salt filtrate. The fourth guinea-pig was kept as control.

On September 27th the three treated animals were reinjected each with 60 milligrams of the same salt filtrate.

One of the treated guinea-pigs was found dead on October 2nd. The control and a second treated animal were found dead on October 3rd, and the last of the treated guinea-pigs was found dead on October 4th. All four animals showing the appearances of sub-acute plague with copious *B. pestis* in the necrotic bubo and the necrotic nodules of the spleen.

From these experiments it appears that in the amounts used, two injections—110 milligrams altogether of salt filtrate—rat organ material has practically no effect in counteracting a previous infection with *B. pestis*.

#### SERIES IV.

In this series therapeutic material was sought from the organs of a guinea-pig which had recovered after two separate inoculations with *B. pestis*.

The history of the animal is as follows:—

On June 19th a guinea-pig was inoculated cutaneously with agar culture of attenuated *B. pestis*. This agar culture (monkey bubo stock) had been growing for 7 days at 37° C.—a period sufficient, as experience has shown, to cause marked attenuation of the *B. pestis*.

For two days after inoculation slow development of bubo was noted, and on the third day the animal was quiet and a little off its feed. The bubo went on enlarging; on June 23rd it was about the

size of a pigeon's egg, but the animal was now lively and fed well. By June 29th the bubo had markedly decreased, and on July 6th a firm nodule not larger than a pea occupied its place. On this day (July 6th) the animal was subcutaneously injected in the other groin (the right) with about  $\frac{1}{2}$  cc. of a turbid emulsion of an agar culture 11 days old of the monkey bubo stock.

On July 13th it showed distinct bubo in the right groin, but was lively and fed well. This bubo did not enlarge, and by August 8th had entirely disappeared, the animal remaining throughout lively and well.

On August 8th the animal was killed. Post-mortem examination showed all viscera perfectly normal, no *B. pestis* or any other microbe discoverable by films or by culture.

The liver and spleen of this guinea-pig were cut out, finely minced and dried at 46° C. in the usual manner, and the powder thus obtained, which was preserved in sterile bottle, will be spoken of hereafter as "guinea-pig therapeutic."

*Experiment 12.*—Four guinea-pigs were inoculated cutaneously on September 18th with a 24 hours' agar culture of monkey bubo stock.

On September 19th all four animals appeared quiet. Three of them were now injected subcutaneously with 62.5 milligrams each of salt emulsion of the above "guinea-pig therapeutic," the fourth being reserved as control.

On September 20th all showed developing bubo.

On September 21st all were quiet but feeding fairly well.

On September 22nd all had marked bubo and were off their feed.

On September 24th two of the treated animals, as also the control, were found dead. The third treated guinea-pig was found dead on September 28th. All four animals had died of typical sub-acute plague.

*Experiment 13.*—On the same date as in the case of experiment 12, viz., September 18th, four rats were inoculated cutaneously with *B. pestis* of precisely the same agar culture as the guinea-pigs.

On September 19 three of these four rats each received subcutaneously 62.5 milligrams of the same salt emulsion of the "guinea-pig therapeutic." The fourth rat being reserved as control.

The control rat and two of the treated rats were found dead on the morning of September 21st, and the third treated rat was found dead on the morning of September 22nd. All four rats died of acute plague.

These two experiments not seeming to promise anything in the way of satisfactory therapeutic agency with this guinea-pig material, no further experiments were made with it.

#### SERIES V.

In this series employment was made of the organs (liver and spleen) of rabbits which had passed through and fully

recovered from plague. The history of two of these rabbits is as follows:—

*Rabbit No. 1.*—On June 8th this rabbit received subcutaneously in the groin 25 milligrams, and on June 15th 44 milligrams, of twice-heated watery filtrate of tested organ prophylactic of guinea-pig stock. The animal remained unaffected. The particular filtrate had proved definitely prophylactic for rats and monkeys.

On June 28th the rabbit received subcutaneously in the neck about half a culture of a 24 hours' agar culture of virulent monkey bubo stock of *B. pestis*.

On July 6th the animal showed an extensive firm tumour, comprising the back of the neck and extending close to the ear. This tumour had opened and was discharging. The animal seemed lively and fed well.

On July 25th there was extensive thickening and ulceration on neck.

On August 11th the ulceration had nearly healed, thickening was almost gone. The animal was lively and was feeding well.

On August 29th there was no trace of previous ulceration or thickening. The animal was quite normal.

On September 18th the rabbit was re-injected (subcutaneously in groin) with half a culture (24 hours' agar) of virulent *B. pestis* (monkey bubo stock).

On September 19th the animal showed slight swelling in the groin and was a little quiet, but it was feeding.

On September 22nd there was no trace of this swelling, and the animal seemed quite normal.

The rabbit was killed on October 5th, that is 17 days after the second injection.

On post-mortem examination all its viscera were found normal, no trace anywhere of swollen glands. The liver and the spleen were prepared in the usual manner: minced and dried at 46° C. for three days, then powdered and dried in sterile bottle for two days at 37° C. This material will be referred to as "Rabbits therapeutic of October 5th."

*Rabbit No. 2.*—Two rabbits were injected on October 12th, subcutaneously in the neck, with agar culture of *B. pestis*. The agar culture (monkey bubo stock) was 18 days old, and each animal received half a culture.

On October 15th both animals were ill, quiet and off their feed.

On October 16th one was found dead with acute plague. The other was ill but not seriously so; it had a big bubo in the region of the neck, was quiet and did not feed well.

On October 18th this animal was still ill. On October 19th it was still a little quiet but was taking some food. On October 24th there remained thickening about the neck, but the animal was distinctly livelier and fed well. From October 26th till October 30th, except for slight thickening in the neck, the animal seemed all right. On November 6th no trace of thickening could be felt, and the animal appeared in all respects normal.

It was killed on this day (November 6th). On post-mortem examination no sign of enlarged glands in the neck or anywhere else could be detected; the viscera appeared normal, except the

spleen was found juicy and slightly enlarged. Neither by film nor by culture could any bacteria be discovered in the liver or in the spleen. These two organs were now cut out and prepared in the usual way for obtaining and preserving the powdered material. This material will be referred to as "Rabbits' therapeutic of November 6th."

*Experiment 14.*—Three guinea-pigs were on October 10th cutaneously inoculated with a 24 hours' agar culture of virulent *B. pestis* (monkey bubo stock).

About half-an-hour afterwards two of the animals received each subcutaneously 50 milligrams of "rabbits' therapeutic of October 5th." The third animal being kept as control.

Next day, October 11th, the two treated guinea-pigs each again received 60 milligrams of filtrate of same therapeutic.

On October 13th the control animal showed big bubo, the treated guinea-pigs having only slight swelling. On October 15th all three animals were found quiet. The two treated guinea-pigs were now re-injected each with 50 milligrams filtrate of same therapeutic.

On October 16th the control guinea-pig appeared very ill, almost dying, whereas the treated guinea-pigs seemed lively, although each had good-sized bubo. These two treated guinea-pigs were now again re-injected each with 50 milligrams filtrate of same therapeutic.

On October 17th in the morning the control animal was found dead, and on post-mortem showed typical sub-acute plague. The treated guinea-pigs, although each exhibiting fair sized bubo, were lively.

On October 18th the bubos of both the treated guinea-pigs had opened spontaneously and were discharging; the animals appeared lively, feeding normally.

On October 20th one of the treated guinea-pigs was found dead; post-mortem showed marked sub-acute plague. The other treated guinea-pig appeared quite lively and was feeding well.

On October 23rd this second treated guinea-pig was found dead, and on post-mortem examination showed sub-acute plague.

*Experiment 15.*—On October 12th three guinea-pigs were inoculated cutaneously with a 24 hours' agar culture of virulent *B. pestis* (monkey bubo stock). Half-an-hour later two of them received each subcutaneously 50 milligrams of salt filtrate of "Rabbits' therapeutic of October 5th," the third being kept as control.

On October 13th the two guinea-pigs were re-injected with like amounts as before of the same material.

On October 15th they were again injected with like amounts of this material. On this day the control guinea-pig showed big bubo, whereas the treated animals showed only slight swelling.

On October 16th one of the treated guinea-pigs and the control animal were found dead. Post-mortem examination showed commencing sub-acute plague in both animals. At this date the second treated guinea-pig appeared lively, though showing bubo. It was re-injected with 50 milligrams of the same material as before. This guinea-pig on October 20th seemed quite lively and

was feeding, but it showed big bubo. It was found dead on the morning of October 22nd. Post-mortem examination revealed extensive sub-acute plague: necrotic bubo, numerous necrotic spots in liver and spleen, and extensive necrotic patches in the lungs, all pervaded with *B. pestis*.

*Experiment 16.*—On October 25th four rats were inoculated cutaneously with a 24 hours' agar culture of virulent *B. pestis* (monkey bubo stock).

About half-an-hour afterwards three of them were injected, subcutaneously, each with 50 milligrams of salt emulsion of "Rabbits' therapeutic of October 5th," the fourth serving as control.

On October 27th, the control animal was distinctly ill. It died in the night with typical acute plague, its bubo and spleen being crowded with *B. pestis*. The treated rats appeared lively.

On October 29th one of the treated rats was found dead. Post-mortem examination showed: hæmorrhagic bubo, few bacilli; spleen not enlarged, bacilli exceedingly scarce, film specimens showing here and there in few places single *B. pestis*; lungs congested, no bacilli to be found in them.

On October 31st a second treated rat was found dead. Post-mortem examination showed: slight bubo with few bacilli; spleen not markedly enlarged with few small groups of bacilli *pestis*; films of the lung juice shewed no bacilli.

The third treated rat remained unaffected and survived.

*Experiment 17.*—On November 2nd four rats were cutaneously inoculated with broth culture of virulent *B. pestis* (rabbits' stock).

On November 3rd three of these rats received each subcutaneously 50 milligrams of salt emulsion of "rabbits therapeutic of October 5th"; the fourth animal being kept as control.

On November 5th the control animal was very ill, and it died in the evening. Post-mortem shewed typical acute plague, organs crowded with *B. pestis*.

On November 6th one of the treated rats was found dead of typical acute plague with crowds of *B. pestis* in the tissues.

On November 7th the other two treated rats were found dead with typical acute plague.

*Experiment 18.*—On November 7th four rats were inoculated cutaneously with same broth culture of *B. pestis* as was employed in previous experiment.

On November 8th three of these rats received each subcutaneously 100 milligrams of filtrate of watery emulsion of the "rabbits' therapeutic of October 5th," the fourth rat serving as control.

On November 10th, in the morning, the control and two of the treated rats were found dead from typical acute plague, with crowds of *B. pestis*.

On November 12th the third rat still remained unaffected, and it survived.

From these experiments with "rabbits' therapeutic of October 5th," it appears that this material possessed some slight therapeutic action. Thus in Experiment 14 on infected guinea-pigs, repeated injection with the "therapeutic" caused a marked delay of the

lethal end, though it failed to prevent death. In Experiment 16 on three plague infected rats a single injection of 50 milligrams of the material modified in two instances the plague that had been induced, in that the spleens of the rats were not enlarged and contained but very few plague bacilli; while the third rat of the series remained quite unaffected by the plague infection to which it had previously been subjected. Similarly in Experiment 18, also on rats, in which 100 milligrams of the "therapeutic" were administered in a single injection 24 hours after cutaneous infection with virulent *B. pestis*, one out of three rats altogether escaped illness. In two, however, of the experiments (15 and 17), the therapeutic action of this material was practically nil.

The seven next succeeding experiments were performed with the material obtained from rabbit No. 2 of November 6th. This was the rabbit which, having passed through distinct plague, without of course lethal result, had been reinjected with large dose of culture of *B. pestis* without causing any disease.

*Experiment 19.*—On November 12th four rats were inoculated cutaneously with broth culture of *B. pestis* (rabbits' stock).

On November 13th three of these rats received each subcutaneously 50 milligrams of salt emulsion of the "rabbits' therapeutic of November 6th," the fourth rat being kept as control.

On November 15th the control rat was ill, and died on the evening of November 16th with typical acute plague. The treated rats appeared at this date unaffected, and they were each reinjected with 50 milligrams of same material. They remained unaffected and survived.

*Experiment 20.*—On November 19th two rats were inoculated cutaneously with same broth culture of *B. pestis* of same stock. These rats received each subcutaneously on November 20th 50 milligrams of salt emulsion of same "rabbits' therapeutic of November 6th." On November 21st they were re-injected with like dose of same material. They remained unaffected and survived.

*Experiment 21.*—On November 19th (at the same time as the rats in Experiment 20) three guinea-pigs were inoculated cutaneously with same broth culture of *B. pestis* of rabbits' stock.

On November 20th two of the guinea-pigs received each subcutaneously 50 milligrams of the same "therapeutic" as in Experiment 20, the third guinea-pig serving as control.

On November 21st the two treated animals were re-injected each with 50 milligrams of same material.

The control animal was found dead on November 28th with sub-acute plague, whereas the two treated guinea-pigs were lively and feeding well; they showed, however, bubo in the groin. By December 6th the bubo had become in each instance transformed into an abscess. These abscesses were opened and cleared of pus. Both animals recovered.

In these three experiments the particular material appears to have acted in a not unsatisfactory sense therapeutically. But it has to be added that the infecting *B. pestis* (broth culture) against

which it was in antagonism could not have been of a virulent type, since the control animals (Experiments 19 and 21) died somewhat later than is customary when the *B. pestis* of high virulence is in question, as was the case with certain experiments of the preceding series. It may be inferred indeed that there exists, as one would suppose from *a priori* reasons, an inverse relation between the effect of the "therapeutic" and the virulence of the infecting *B. pestis*; in other words, that the greater or lesser therapeutic effect of a given material depends largely on the lesser or greater virulence of the infecting *B. pestis*.

*Experiment 22.*—On December 10th three rats were inoculated cutaneously, in each instance with the sanguineous juice of the hæmorrhagic bubo of a rat which had died in 48 hours of acute typical plague, the bubo juice being crowded with *B. pestis*. Two of these rats received each on December 11th 75 milligrams of salt emulsion of the "rabbits' therapeutic of November 6th," the third rat being kept as control.

On December 12th the control rat and one of the treated rats were found ill.

On December 13th the control rat was found dead of typical acute plague with abundance of *B. pestis*, and one of the treated rats was dying. After death of this animal examination showed hæmorrhage at the seat of the therapeutic injection, but no *B. pestis* could be discovered either in the spleen, which was not enlarged, or in the blood.

The second treated rat was found dead on December 14th. This animal showed also hæmorrhage at the seat of the therapeutic injection, but the spleen was not enlarged, and no *B. pestis* could be discovered in it or in the blood.

*Experiment 23.*—On January 1st three rats were inoculated each cutaneously with 24 hours' agar culture of virulent *B. pestis* (spleen of a plague guinea-pig).

On January 2nd two of these rats received each 82 milligrams of watery filtrate of "rabbits' therapeutic of November 6th," the third being kept as control.

On January 3rd the control rat was found very ill, but the treated rats were only slightly affected.

On January 4th the control and one of the treated rats were found dead. In both of these there was evidence of acute typical plague, with abundance of *B. pestis* in the enlarged spleen. The second treated rat appeared still slightly off its feed and a little quiet. This animal lived till January 8th, when it died. Post-mortem examination showed necrotic bubo, but no *B. pestis* in it; the spleen was not enlarged, but a few small groups of *B. pestis* were discovered in it by film specimens. This rat, then, did die of plague, though of a modified attenuated type.

*Experiment 24.*—On February 14th four rats were inoculated each cutaneously with spleen juice of a rat which had just died of acute (40 hours duration of illness) typical plague, the spleen being much enlarged and crowded with *B. pestis*.

On February 15th three of these rats received each 100 milligrams of the clear watery filtrate of "rabbits' therapeutic of November 6th," the fourth being kept as control.

The control rat died in the evening of February 16th, that is to say, in about 56 hours. The post-mortem examination showed hæmorrhagic bubo and enlarged spleen, both organs being packed with *B. pestis*. The treated rats showed very little alteration, being fairly lively and still feeding. All three survived.

*Experiment 25.*—On February 18th six guinea-pigs were inoculated each with 48 hours' agar culture of virulent *B. pestis* (guinea-pig spleen).

On February 19th five of these guinea-pigs received each 100 milligrams clear watery filtrate of "rabbits' therapeutic of November 6th." The sixth being kept as control.

On February 23rd all six animals had bubo. The control guinea-pig was found dead on the morning of February 25th, showing sub-acute plague of pronounced kind: bubo, liver, spleen, and lungs containing necrotic nodules and patches crowded with *B. pestis*.

On February 26th one of the treated guinea-pigs was found dead of sub-acute plague.

On March 1st two further treated guinea-pigs were found dead of sub-acute plague.

On March 4th the remaining two animals showed instead of the initial bubo a small firm nodule; otherwise the animals appeared lively and feeding well. On March 11th all traces of this nodule were gone; the animals appeared normal. They were kept till the end of March, when they were killed. Post-mortem showed nowhere any abnormal condition; films and cultures showed no microbes of any kind.

From these last two experiments it appears that the filtrate of a watery emulsion of the tissues in doses of 100 milligrams injected into rodents 24 hours after infection of these animals with virulent *B. pestis* had a marked therapeutic effect; and that this effect was more pronounced in the case of the rat than in that of the guinea-pig. This more promising result, be it noted, was obtained by agency of material from the liver and the spleen of a rabbit which after having passed through a definite attack of plague had effectively withstood a second injection of a large dose of virulent *B. pestis*.

It may be that there is here basis for securing a satisfactory therapeutic agent from cases of naturally acquired plague. But obviously there are points which require to be made the subject of further experiment.

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## APPENDIX B, No. 2.

OBSERVATIONS on some of the DEFENSIVE MECHANISMS of the BODY against the PYOGENIC COCCI; by F. W. ANDREWES, M.D., F.R.C.P., and M. H. GORDON, M.D., B.Sc.

In accordance with the instructions of the Board that we should extend our joint investigation of staphylococcus and streptococcus infections with special reference to methods of inhibiting the injurious activities of pathogenic micro-organisms of this class when they gain access to the animal body, we have begun to investigate the defensive mechanisms possessed by the normal body against invasion by the pyogenic cocci, and the methods which may be employed to reinforce the natural means of defence. Such a field of inquiry, covering as it does a large part of the subject of immunity, is a very extensive one, and in the present preliminary stage of the investigation we have been compelled to confine our attention to certain of the means of defence possessed by the normal body, and to test in the first place the methods which are available for measuring such defensive powers.

*Present state of knowledge as to Immunity against the Pyogenic Cocci.*

Notwithstanding the fact that the pathogenic staphylococci and streptococci are on all hands admitted to occupy a foremost place among the bacteria which cause disease in man, the machinery upon which the living body depends for resistance against invasion by these micro-organisms has only of recent years received the attention it deserves. This is largely due to the complex nature of the processes involved, and the consequent difficulty of attacking the problem experimentally. Not only does the requisite investigation entail a separate study of the microbe and of its host, but, above all, of the inter-action between the two. Both the factors concerned are living organisms and as such are liable to individual variation; but while the micro-organism can be cultivated outside the body, and the upper and lower limits of its capacity for producing disease can be defined, the resisting powers of the host, still more complex, unstable, and "resultant" in their character, are far more difficult of definition. In recent years, however, the labours of Buchner, Pfeiffer, Behring, Metchnikoff, Bordet, Ehrlich, Wright and others have enabled us to obtain some insight into the factors upon which successful resistance depends and have supplied the basis of a new branch of pathology known as immunology.

It is now well recognised that the body reacts against bacteria in a number of different ways. Where the bacterium produces a powerful soluble poison (as in diphtheria and tetanus) the body responds by producing a chemical antidote or "antitoxin." Few of the pathogenic bacteria, however, form such soluble poisons;

in the majority the poison appears to reside in the body of the bacterium itself (endotoxin) and here the reaction of the host is directed against the actual bacterium rather than against its products. In the light of our present knowledge there are two chief means by which the bacterium is attacked. One is by the production of substances termed lysins which are present in the serum and attack and destroy it directly, apart from mechanical ingestion by special cells; this process is known as "bacteriolysis," and it has been noted especially in the reaction against the cholera vibrio and the typhoid bacillus. The other, and apparently the commoner means, is by the direct activity of the leucocytes, which engulf the bacteria, destroy them after ingestion, and have therefore been termed "phagocytes." The most active phagocytes are the polynuclear leucocytes of the blood, but endothelial cells have been shown to share the power. In recent years, Sir Almroth Wright has shown that phagocytic power is to a large extent conditioned by the preliminary influence upon the bacteria of substances present in the serum which he terms "opsonins." It would seem that unless the bacteria are first acted upon by these substances, phagocytosis does not take place.

We may thus recognise at least three means of defence possessed by the body against invading bacteria. (1) A chemical antidote may be produced, neutralizing the bacterial poison, but not attacking the bacterium itself; this is *chemical* or "*antitoxic*" *immunity*. (2) Substances may be formed capable of destroying the bacteria apart from direct cellular intervention; this is "*Bacteriolytic*" *immunity*. (3) The bacteria may be destroyed by special cells of the body; this is "*phagocytic*" *immunity* or, since the intervention of opsonins plays an important part in the process, "*Opsonic*" *immunity*. Against any individual species of bacterium any or all of these means of defence may be called into play, according to the nature and properties of the invading organism, but it is now generally held that the phagocytic means of defence is that which is most widely employed. It may be, however, that other machinery for defence exists, not yet apparent in the imperfect state of our knowledge.

With regard to defence against the pathogenic staphylococci and streptococci it may be said that there is no sufficient evidence that they form soluble poisons. Cultures in which they have been growing, when filtered through porcelain, yield a sterile filtrate which is almost, or quite, devoid of toxic power. Some claims have indeed been made that streptococci produce a soluble poison but, on the whole, the balance of evidence goes to show that the poisonous powers of all the pathogenic cocci are bound up in the bacterial bodies themselves. Antitoxic immunity against them is therefore not demanded, and is in fact not known to exist.

It is commonly believed that bacteriolytic immunity plays little or no part in defence against these cocci. Previous observers are practically unanimous in declaring that the serum of man and animals, normal or immunised, is devoid of any bactericidal action upon cocci. Nuttall failed to get evidence of any bactericidal effect exerted by normal serum on *Staphylococcus aureus* (*Zeitschrift f.*

Hygiene, Vol. IV, 1888, pp. 353-394). Wright and Windsor (Journal of Hygiene, 1902, p. 397), who repeated these experiments, came to the same conclusion, and also obtained evidence which led them to infer that immunisation of human beings by the subcutaneous injection of sterilised cultures of the staphylococcus does not confer any bactericidal power upon the serum. In the "Lancet," 1902, I., p. 874, Wright declares that he found no evidence of any definite bactericidal effect upon staphylococci either in normal serum, or in that of patients suffering from furunculosis, before and after inoculation with killed cultures of the coccus. Neisser, in an article contained in Ehrlich's "Studies on Immunity" (English Translation, 1906, p. 349), also states that experiments on rabbits and goats, extending over several years, were unsuccessful in producing a serum bactericidal *in vitro* against *Staphylococcus aureus*, though he admits that a serum can be obtained which appears to exert such an action *in vivo*. Similar facts are claimed as regards streptococci (*vide* Denys: Centralblatt f. Bakt. Abth. I, Vol. XXIV, 1898, pp. 685-691).

The only evidence which has hitherto been forthcoming as to the means of defence against the pyogenic cocci, refers such defence to phagocytic action. In particular, it has been shown that the serum of animals immunised against *Staphylococcus aureus* contains an increased amount of the substance which prepares this coccus for phagocytosis (opsonin). The first research of Wright and Douglas, in which the existence of opsonin was demonstrated (Proceedings of the Royal Society, Vol. 72, No. 483, Oct. 1903), was in fact carried out with *Staphylococcus aureus*, and Bulloch and others, who repeated and confirmed these observations, used this same micro-organism.

There has thus arisen a generally accepted opinion that the means of defence possessed by the animal body against the pyogenic cocci is essentially a phagocytic defence, dependent on the presence of an opsonic substance in the body fluids which prepares the cocci for phagocytosis. During the last two or three years the "opsonic index" has come to be regarded by many as the essential criterion by which the condition of the bodily defence is to be judged.

Certain facts have, however, been brought to light showing that the bacterium is not merely a passive pawn in the game. Many observers in America have repeated and confirmed the results obtained by Wright and his fellow workers; they have also extended the observations, and in this respect the work of Rosenow is of especial note. Studying the pneumococcus in relation to phagocytosis and opsonic power, Rosenow was able to show that there exists an inverse ratio between the virulence of a given strain of pneumococcus and its capacity for being taken up by phagocytes. Pneumococci of high virulence could not be taken up at all; as their virulence lessened on sub-culture, the phagocytes became able to engulf them in presence of opsonin: in one strain which had been under cultivation for a long time and which had totally lost its virulence, phagocytosis was freely seen even in absence of opsonin. These facts suggest that the "virulence" of the coccus—i.e., its capacity for invading the body and setting up

disease—is closely related to its power of defying the phagocytic mechanisms employed in bodily defence. A phagocytic index representing a fair degree of immunity against pneumococci of low or moderate virulence might well co-exist with but feeble powers of resistance against the highly virulent organism.

Further, the almost exclusive importance which has of late been attached to the opsonic index as a gauge of immunity, at least in this country, clearly rests upon the belief that no other means of defence than phagocytosis is worth consideration in respect to the pyogenic cocci. Upon the evidence so far forthcoming, this belief would appear justified. Yet, could it be shown that other means of defence were less inappreciable than has hitherto been supposed, an explanation would be afforded of certain curious discrepancies attending the relation between recovery from coccal infections and the opsonic index. It is certain that careful inoculation with a vaccine of sterilised staphylococci is followed by a rise in the opsonic index, but it is no less certain that the cure of chronic furunculosis (and the permanence of the cure) by staphylococcal vaccines, does not so closely correspond with the behaviour of the opsonic index as to point to this as the sole factor concerned. It may well be that the methods which have hitherto been employed in estimating the bactericidal power of the serum against the pyogenic cocci have not been suitable for the detection of its presence in small degree—a degree soon exhausted, yet perchance of no small moment in the earlier stages of infection. It may be, again, that the cure of the patient depends not only on an increased production of opsonin, but on an increased production of phagocytes. Since, too, it is not unlikely that opsonin may be a product of the phagocytes themselves, it is possible that in the course of immunisation some change may be wrought in the powers of the leucocytes in this respect.

It is certain that further knowledge is required as to these and many kindred points, before our knowledge of immunity against the pyogenic cocci can be said to rest upon an assured basis.

#### *Scope of the present Report.*

In the present inquiry, therefore, our attention has mainly been directed to the host. The object, broadly speaking, was to ascertain the principal means whereby the living body defends itself against invasion by pathogenic micrococci. An immense amount of work on opsonins has been carried out during the past few years by many observers. We have therefore, for the most part paid little attention to this branch of the subject, not because we in any way underrate its importance, but rather because we believed that its obvious importance had perhaps unduly thrown other aspects of defence into the shade. We thought it advisable to limit our observations in the first place to one micro-organism, and selected *Staphylococcus pyogenes aureus* as the best adapted for our purpose. The animal which we chose for study as regards its inter-actions with the staphylococcus was the rabbit—an animal neither too susceptible nor too refractory to the coccus in question.

Observations have been made both on normal rabbits and on those protected by previous treatment with the staphylococcus. The reactions which we have chiefly studied have been those presented by the blood. These have been examined *in vitro* in blood withdrawn from one of the small veins of the ear and maintained outside the body at 37° C. with due precautions to avoid clotting. Further, we have commenced to study the reaction *in vivo* by introducing a known quantity of the staphylococcus into the circulation and observing the phenomena which could be detected in the living blood-stream. We desire to acknowledge our indebtedness to Mr. P. C. Laws, who has throughout rendered us valuable voluntary assistance.

## SECTION II.

### OBSERVATIONS ON THE BACTERICIDAL POWERS OF THE BLOOD UPON STAPHYLOCOCCUS PYOGENES AUREUS.

#### A.—Experiments carried out by Wright's Method.

We commenced our observations by testing for ourselves the bactericidal powers of the blood upon *Staphylococcus pyogenes aureus*. Of the various methods which have hitherto been used to this end, that devised by Wright appeared to us the best; we therefore selected it for our first experiments.

In this method, as described by Wright (Proc. Roy. Soc., Vol. LXXI., No. 468, 1902), a series of decimal dilutions is prepared from a culture of the test organism. Small quantities of the serum are mixed with equal volumes of the successive dilutions in separate capillary pipettes, which are then sealed and incubated at body temperature for a standard time (18 to 24 hours). The contents of the pipettes are now expelled on to the surface of agar tubes, or some other suitable culture medium, and the life or death of the organisms determined on incubation. It is thus ascertained how far up the series of dilutions the killing power extends, and, if the number of organisms in the original culture be known, a numerical expression of the bactericidal power of the serum is forthcoming. It is determined, in fact, how many of the bacteria a given volume of serum can kill, in given time, and at a given temperature.

We tested, first, the bactericidal power of the blood as a whole, before examining that of its various constituents. To do this it was necessary to prevent clotting, which was attained by mixing with citrated saline solution. If blood was taken from a person or animal at hand in the laboratory, we used the citrated saline solution for preparing the decimal dilutions of the culture, and then brought the freshly drawn blood directly into contact with this citrated suspension of the micro-organisms. When the blood had to be brought from a distance, it was mixed at once with an equal volume of citrated saline solution, conveyed to the laboratory, and tested against dilutions of the micro-organism prepared with ordinary saline solution.

The culture used in these preliminary experiments was a 24 hours' broth culture of *Staphylococcus aureus*, and dilutions of this were prepared, ranging from 1 in 10 up to 1 in 100,000,000. Thus seven or eight separate pipettes were used in testing every sample of blood upon the staphylococcus.

### Results.

#### (a) HUMAN BLOOD.

*Experiment I.*—Blood from a normal adult was tested against dilutions of the staphylococcus culture ranging from 1 : 100 to 1 : 100,000,000. About 1/100 cc. of blood was mixed with an equal volume of each dilution. A control experiment was done in which ordinary nutrient broth was substituted for the blood. After 24 hours' incubation at 37° C. the contents of the pipettes were expelled on to agar tubes and incubated. Growth occurred both with the blood and in the control with broth, up to the 1 : 10,000,000 dilution ; i.e., there was no evidence that the blood exerted any bactericidal action upon the cocci.

*Experiment II.*—Repetition of the preceding experiment. Both with blood and broth, growth occurred up to the 1 : 1,000,000 dilution, confirming the previous result.

*Experiment III.*—Repetition of the same experiment with the blood of another adult : contact was for 17 hours at 37° C. Again, growth occurred both with the blood and with broth up to the 1 : 1,000,000 dilution.

*Experiment IV.*—The same experiment was carried out with blood from the cadaver of an adult dead of malignant endocarditis. Again, no evidence was obtained of any bactericidal power upon the staphylococcus, whereas a control experiment carried out with the same blood upon the typhoid bacillus showed it to exert a strong bactericidal action—all dilutions from 1 : 1,000 to 1 : 10,000,000 being sterilized.

*Experiment V.*—The test was made with the blood of a patient in a pyæmic condition with metastatic abscesses due to the *Staphylococcus pyogenes aureus*. Control experiments were at the same time done with normal human blood and with broth. In all three cases growth occurred up to the highest dilution employed, but the colonies were less abundant in the case of the patient's blood. It thus appeared possible that the blood of a person suffering from staphylococcus infection might possess either a slight bactericidal effect upon the staphylococcus, or might inhibit its growth to some extent. The effect, as tested by this method, was but slight, and the influence of agglutination of the cocci could not be excluded as regards the number of colonies which developed.

#### (b) RABBIT'S BLOOD.

Tests were carried out in the same fashion with the blood of normal rabbits, and of a rabbit strongly immunised against

*Staphylococcus aureus* by repeated intravenous injections of the cocci killed by heat.

*Experiment I.*—Blood from three normal rabbits was tested, contact being, as before, for 24 hours at 37° C. No evidence of any bactericidal action upon the *staphylococcus* was obtained.

*Experiment II.*—The blood of a normal rabbit was tested side by side with that of a strongly immunised rabbit; in neither case was any evidence of bactericidal action to be found.

### (c) ANTI-STAPHYLOCOCCAL SERUM FROM THE HORSE.

By the kindness of Dr. C. J. Martin and Dr. A. MacConkey, of the Lister Institute of Preventive Medicine, we were furnished with fresh serum from a horse highly immunised against *Staphylococcus pyrogenes aureus*, and from a normal horse, no antiseptic having been in either case added to the serum. We are further indebted to Sir John McFadyean for a supply of fresh horses' blood, from which we derived still living leucocytes.

Experiments with these materials were carried out by us in duplicate, each observer working independently.

*Experiment I.*—In this we compared the direct bactericidal action of normal and immune horses' serum in absence of leucocytes. Neither of us was able to obtain evidence of direct bactericidal action, even with the immune serum; indeed, it was clear that considerable multiplication of the cocci took place under the conditions of the experiment. Thus, to give one instance, 1 cc. of the final dilution of the broth culture (1:100,000,000) yielded, when plated out in agar, 94 colonies; yet 1/100 cc. of this dilution, after 20 hours' contact with an equal volume of the anti-staphylococcus serum at 37° C. yielded more than 100 colonies of the coccus. In one experiment the immune serum appeared to have a slight advantage over the normal serum, the colonies arising being slightly less numerous with the higher dilutions, but the difference was trivial, and not always apparent.

*Experiment II.*—We next compared the relative bactericidal action of the two sera in presence of living leucocytes. The blood of a normal horse was received into citrated saline solution, brought straight to the laboratory and centrifugalised, the corpuscles being washed twice. The opsonic index of the immune serum, as compared with the normal serum, was 1·8. The broth culture of the coccus was filtered before making dilutions from it; this reduced the number of cocci in it to 20 millions per cc.

Equal parts of serum, washed corpuscles, and dilute broth culture were mixed together and treated as in the preceding experiments.

Again, the method failed to reveal any important degree of bactericidal action on the part of either serum; our independent sets of experiments were alike in this result. Nevertheless the immune serum showed a slight advantage over the normal in

respect to the number of colonies which developed. As an example of this we subjoin the data of the most consistent result obtained; the table shows the growth from 1/100 cc. of the dilutions of the culture of staphylococci, under the conditions set forth.

TABLE I.—EFFECT of NORMAL and IMMUNE HORSE'S SERUM, in CONJUNCTION with LIVING LEUCOCYTES upon STAPHYLOCOCCUS PYOGENES AUREUS.

Dilution of the Culture.	1 : 100.	1 : 1,000.	1 : 10,000.	1 : 100,000.	1 : 1,000,000.
<i>Control</i> : 1/100 cc. of dilution transferred directly to agar tubes.	Confluent growth.	Discrete colonies.	A few colonies.	Two colonies.	Sterile.
<i>Normal serum</i> + corpuscles + coccal dilution, of each 1/100 cc.: after 20 hours' incubation together.	Confluent growth.	Confluent growth.	Confluent growth.	Three colonies.	Sterile.
<i>Immune serum</i> + corpuscles + coccal dilution, as before.	Confluent growth.	Confluent growth.	Discrete colonies.	Sterile.	Sterile.

Here, with the 1 : 10,000 dilution, multiplication had evidently occurred in presence of both sera, though more with the normal than with the immune. With the 1 in 100,000 dilution, the absence of growth with the immune serum may have been mere chance, seeing that only two colonies were present in the control.

*Experiment III.*—This was a repetition of Experiment II., and was also done in duplicate. The results were just as before.

*Experiment IV.*—Experiments were also carried out with normal and immune horses' serum in bulk, instead of in capillary pipettes; measured amounts of a diluted broth culture were added to equal measured amounts of the sera, and the number of cocci before and after 24 hours' incubation was ascertained by plate cultures. Multiplication occurred in both sera, but more so in the normal than in the immune serum.

#### *Conclusions from the preceding Experiments.*

With the capillary pipette method of Wright, therefore, we failed to obtain positive evidence as to any bactericidal effect exerted upon *Staphylococcus pyogenes aureus* by (1) human blood; (2) normal and immune rabbits' blood; (3) normal and immune horses' serum; or (4) normal and immune horses' serum, in presence of living leucocytes. These results, it may be added, are in complete accord with those of previous observers.

But, notwithstanding the fact that parallel tests made upon *Bacillus typhosus* showed the method to be excellent for determining differences in the bactericidal values of different bloods for this organism, we were not satisfied that our negative results with the staphylococcus were conclusive. The number of organisms present after 24 hours' incubation with a blood or serum is the resultant of two opposing factors—(a) a tendency to destruction of the organism by anti-bodies in the blood, and (b) a tendency to multiplication of the organisms. The result must in all probability depend upon which of these opposing factors gets the upper hand during the early hours of contact. There is a presumption that a given amount of serum can only destroy a limited number of bacteria; if the number of bacteria present is in excess of this limit, the survivors will be in a position to multiply, for the bactericidal substance will all have been used up. It is plain that Wright's capillary pipette method, as we employed it, would fail to detect lesser degrees of bactericidal action occurring only during the earlier hours of contact and later compensated by multiplication of the surviving organisms.

Accordingly, we proceeded to enquire experimentally into the subject with the view of devising a more delicate and suitable method, whereby a definite and more conclusive answer might be obtained to the question whether or not blood exerts, *in vitro*, a bactericidal action upon *Staphylococcus pyogenes aureus*.

#### B. *Experiments Carried out by Determining the Rate of Bacterial Mortality or Multiplication (as the case may be).*

In attempting to obtain a reliable working method for testing the lesser degrees of bactericidal value possessed by blood or other fluid, the first condition is to find a means whereby a reasonably constant number of the living bacteria may be added to a definite volume of the fluid to be tested, so that the test may be repeated under conditions as closely similar as possible. Secondly, it is needful to find means whereby a definite and uniform, though small, quantity of the mixture may be from time to time withdrawn for examination as to its content of living bacteria.

The procedure generally in vogue in bactericidal experiments is to carry them out in small test-tubes in which definite quantities of the fluid to be examined are brought into contact with a suspension of the given micro-organism. The tubes are kept at constant temperature, and the fate of the micro-organisms is determined by withdrawing at intervals a platinum loopful of the mixture and preparing a plate cultivation therewith.

It is commonly assumed that a platinum loop always removes approximately the same amount. As a matter of fact, although we used the platinum loop in a few of our experiments, we find the variations considerable. By careful weighings carried out on a delicate chemical balance, accurate to 1/10 milligramme, we found that the amount of water which could be dipped up with

a given loop varied from 0.6 to 0.9 milligrammes. In another set of weighings with the same loop the variations were from 0.8 to 1 milligramme. The graduated capillary pipette is a far more accurate measure than the loop, and we have therefore preferred it in the method adopted, though we have found it possible to get reasonably accurate results with the loop in some cases.

The method which we have employed may, for convenience, be termed the "*Mortality-rate method*." It is in no way novel in its principle, which consists in determining the number of living bacteria in a given mixture from hour to hour. The chief feature is the introduction of an approximately uniform number of living micro-organisms into 1 cc. of serum, blood, &c., and the consistent and successful results which the method which we shall now describe, has yielded in our hands depend entirely on attention to minute details of technique. We propose, therefore, to give these as fully as possible.

### *Description of the Method.*

Sterility of all diluting fluids, vessels, pipettes, &c., is an essential, and is here pre-supposed; we shall not refer to it again.

#### *I. Preparation of a standard bacterial emulsion—*

- (a) A surface agar culture is required which has been incubated for 24 hours at 37° C.
- (b) A standard platinum loop is kept at hand, of such size that it holds, when not overcharged, approximately 2 milligrammes of the bacterial growth.
- (c) Two cc. of normal (0.9 per cent.) saline solution are measured into a small test tube, plugged with cotton wool.
- (d) One standard loopful is removed from the surface of the agar culture, and thoroughly mixed with the 2 cc. of normal saline. It is easy to procure a uniform suspension, free from visible clumps.

This constitutes the "*standard bacterial emulsion*," which contains 1 milligramme of bacteria per cc. It is to be noted that slight deviations in the amount of growth removed by the loop are immaterial at this stage, for the actual number of living bacteria present in the emulsion will be revealed by the count to be made at the commencement of the experiment proper, and the variations found in the later hourly counts can be stated as percentages of this.

*II. Dilution of the Standard Emulsion.*—As the result of a series of experiments, it was found that a convenient quantity of the standard emulsion for admixture with 1 cc. of defibrinated blood or other fluid is 0.00001 cc. This amount may be obtained by means of the 1:10 pipette (white counter) of an ordinary Thoma-Zeiss hæmocytometer. Three successive decimal dilutions of the standard emulsion are carried out (using normal saline as the diluent), viz., 1:10, 1:100, 1:1000. The dilutions are

conveniently made in the wells of an artist's porcelain palette. A measured 0.01 cc. of the thousandfold dilution will give the amount required.

III. *The bactericidal experiments* are carried out in small stout test-tubes made from glass tubing of 10 mm. diameter, and about 100 mm. in length. One end is sealed and slightly blown out into an expansion holding 1 cubic centimetre. The free end is plugged with cotton wool. A number of such tubes can be prepared and kept in stock.

A number of 1 cc. pipettes is required for measuring out the fluid to be tested; these may be preserved in a metal box. One cc. of the fluid or fluids to be tested or compared is measured into each of the requisite number of tubes.

To each tube is now added, by means of a 0.01 cc. pipette (= 10 cubic millimetres), this amount of the thousandfold dilution of the standard bacterial emulsion, and thorough admixture is effected by rotating the tube between the palms. In measuring out these small amounts of fluid by pipette, it is of advantage to have ready a number of strips of sterile filter paper in a suitable metal box; with these any excess of fluid can be removed from the end of the pipette, both at this stage and in the subsequent removal of small measured quantities for making plate cultivations.

As soon as the mixtures have been made, a preliminary 0.02 cc. is withdrawn from each tube for plate cultures in the manner about to be described, and the tubes are then placed in a water bath maintained at 37° C. or placed in a rack in an incubator at this temperature. It is advantageous to warm up the tubes of fluid to body temperature before making the mixture.

IV. *Mode of preparation of plate cultures from the mixtures.*—It was determined by experiment that the best amount of the mixture for a plate cultivation, if the preceding ritual has been duly carried out, is 0.02 cc. (= 20 c. mm.). This should yield, at the outset of the experiment, a number of colonies easily counted in a plate culture, usually between 100 and 150. The pipettes which we have used were specially made for us by Lautenschläger, of Berlin, and have proved accurate and easily used. Several such pipettes should be in readiness for an experiment.

Agar tubes are melted and cooled down to between 45° and 50° C., and while the cotton wool plug is removed by an assistant, 20 c. mm. of the mixture is transferred with the pipette to the upper layer of the agar. The plug is replaced, the tube rotated between the palms, and the plate poured. It is best that the Petri dishes used for the plate cultures should be previously warmed, so as to avoid too rapid setting of the agar.

The making of agar plates is carried out at the commencement of each experiment, and thereafter at hourly intervals, or at such intervals as may be desired. Before withdrawing the 20 c. mm., the tube containing the mixture should in each case be well agitated by rotation between the palms. The colonies which arise in the plates are counted after 24, or better after 48 hours' incubation at 37° C.

V. *Mode of obtaining defibrinated blood.*—As will be seen later, the substances which we have tested as regards bactericidal power are numerous, but amongst the most important was the blood, and this it was necessary to defibrinate. The method adopted for obtaining defibrinated blood from the rabbit was as follows:—The ear of the animal, previously shaved, was sterilised by scrubbing, first with soap and water, then with izal (1 in 200), and finally with alcohol. The marginal vein was then obliquely divided, and the blood collected in a short test-tube, with a smooth, straight lip, and furnished with a cotton wool plug. The blood was defibrinated, as it flowed, by gentle agitation with a sterile wire bent into a loop at its extremity. Rather more than 1 cc. of such defibrinated blood was, as a rule, sufficient, but 3 or more cc. could be obtained without difficulty. The wound in the vein was then closed with cotton wool, held in place by a clip, till hæmorrhage had ceased.

It will be observed that, in this method, the amount of blood employed is as small as can conveniently be dealt with under the circumstances, viz., 1 cc.; that the introduction of the requisite amount of bacterial emulsion does not entail dilution of this sample by more than 1 per cent.; and that the quantity withdrawn on each occasion for culture is not more than about 2 per cent. of the whole.

Experience of the method has shown us that, after a little practice, it is possible to repeat the test conditions with remarkable nearness, as may be seen by noting the similarity with regard to the number of cocci introduced at the starting point in many of the later experiments.

The number of cocci present in the mixture is so small that we believe the fallacy introduced by possible agglutination is of little moment, though we shall consider this question at a later stage.

#### RESULTS OBTAINED BY THE "MORTALITY RATE" METHOD OF DETERMINING BACTERICIDAL ACTION.

Before describing the results obtained with blood, serum, &c., it may be well to refer to the outcome of experiments upon *Staphylococcus pyogenes aureus*, as to the influence of distilled water and of various organic and inorganic substances dissolved in distilled water. These experiments served, in the first place, as tests whereby we could judge of the reliability of the method; in the next place, they afforded information as to the physical action of water and other substances upon the coccus whose behaviour we were investigating, and, in the third place, they gave important indications as to the nature of the "pabulum" upon the presence of which multiplication depended. In all our bactericidal experiments we have been careful to bear in mind the distinction between death due to absence of suitable pabulum and destruction by specific anti-bodies. These preliminary experiments are, hence, of no small importance in view of those which follow.

In all cases the experiments were carried out at 37° C.

## A. PRELIMINARY EXPERIMENTS WITH WATER AND SOLUTIONS OF SIMPLE SUBSTANCES.

(1) *Distilled water*.—The effect of distilled water upon *Staphylococcus aureus* at 37° C. is seen in the following table.

(*Note*.—In this and all succeeding tables, unless the contrary is expressly stated, the figures signify the number of colonies developing in an agar plate inoculated with 20 c.mm. of the mixture of staphylococcus suspension with the fluid tested.)

TABLE II.—EFFECT OF DISTILLED WATER ON STAPHYLOCOCCUS AUREUS at 37° C.

—	Experi- ment I.	Experi- ment II.	Experi- ment III.	Experi- ment IV.	Experi- ment V.
At start ... ..	275	71	37	165	105
After 1 hour ...	260	19	6	—	—
After 2 hours ...	88	2	1	0	2
After 3 hours ...	8	0	0	0	0

It is clear that the staphylococcus rapidly perishes in distilled water at 37° C. In four out of five experiments it had completely disappeared after three hours at this temperature. We found, however, that the rate of disappearance varied somewhat in different samples of distilled water, and experiments were instituted to trace the reason for this.

Three samples of distilled water were selected for testing :—

*Water 1* was an exceedingly pure sample of ammonia-free distilled water which had been prepared for another purpose by Dr. Hurtley in the chemical laboratory of St. Bartholomew's Hospital.

*Water 2* was a water roughly distilled in the pathological laboratory without any special regard to cleanliness of apparatus.

*Water 3* was ordinary distilled water from the stock vessel in the public health laboratory, procured from a chemical dealer.

(*Note*.—This particular set of experiments was carried out in a simplified but somewhat less accurate way, using a standard platinum loop for inoculating the surfaces of sloped agar tubes. The standard staphylococcal emulsion was diluted 50 fold, and 10 c.mm. of this dilution was added to 1 cc. of each water. A single loopful of the mixture was withdrawn for each culture.)

The result of the experiment is seen in the following table:—

TABLE III.—EFFECT of THREE DIFFERENT SAMPLES of DISTILLED WATER on STAPHYLOCOCCUS AUREUS at 37° C.

—	Water 1.	Water 2.	Water 3.
At start ... ..	36	32	32
After 1 hour ... ..	10	26	0
„ 2 hours ... ..	3	9	0
„ 3 „ ... ..	0	7	0
„ 4 „ ... ..	0	2	0
„ 5 „ ... ..	0	2	0

The results are seen to be uniform and consistent for each water, but show a marked difference in the rate of mortality in the different waters. No. 3 was already sterile in an hour and No. 1 in three hours, while No. 2 still showed some surviving cocci at the end of five hours.

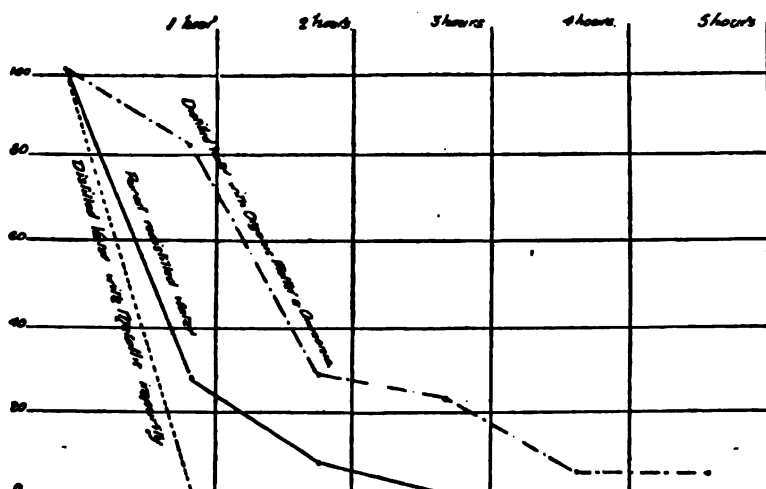
We next submitted the waters to Dr. Hurtley for chemical analysis, and he was so kind as to devote considerable attention to the matter. The result of his investigations is seen in Table IV. subjoined for comparison with Table III.

TABLE IV.—CHEMICAL CHARACTERS of the THREE DISTILLED WATERS USED in the PRECEDING EXPERIMENT.

—	Water 1.	Water 2.	Water 3.
Acidity in terms of $N\frac{1}{10}$ K.H.O. per 50 cc. of water.	·1 cc.	·2 cc.	·5 cc.
Free ammonia by Nesler's reagent	0	·0002 per litre	0
Organic matter by permanganate...	0	Large trace	0
Metallic impurity ... ..	0	0	Discolouration with $H_2S$ to iron.

It is thus seen that No. 1 is an exceptionally pure distilled water; No. 2 contains organic matter and free ammonia; while No. 3, though free from organic impurity, is an acid water containing metallic impurity. These facts probably explain the differences in bactericidal action seen in Table III. The differences are most clearly seen by the aid of a diagram.

DIAGRAM I.—PERCENTAGE REDUCTION of STAPHYLOCOCCI in THREE SAMPLES of DISTILLED WATER.



As it was a matter of some surprise to find that such small traces of organic impurity could influence the death-rate of cocci in distilled water, a further experiment was carried out to ascertain how small an amount of organic matter could exert this influence. The experiment was carried out by the loop method exactly as in the preceding case. Solutions of Witte's peptone were made up in the very pure water (No. 1) used in the foregoing experiment. All glass vessels used were freed from organic matter with strong sulphuric acid. One gramme of peptone was dissolved with gentle warmth in 250 cc. water. This gave an opalescent fluid which filtered clear, but the filtration must have removed some of the organic matter, so that the solutions prepared were really weaker in organic matter than is stated below. Decimal dilutions of the peptone solution were prepared of strengths calculated from 1 in 100,000 to 1 in 1,000,000,000. They were then sterilized, and after two days the tests were made as in the preceding experiment, with the following result:—

TABLE V.—EFFECT of TRACES of PROTEID in DELAYING the DEATH of STAPHYLOCOCCI in DISTILLED WATER.

—	Peptone 1:100,000.	Peptone 1:1 million.	Peptone 1:10 million.	Peptone 1:100 million.	Peptone 1:1,000 million.	Pure Water.
At start ...	196	185	188	184	199	100
After 1 hour	168	215	135	180	91	151
" 2 hours	137	161	50	119	28	70
" 3 "	91	125	12	64	4	24
" 4 "	70	58	1	17	0	2
" 5 "	102	57	0	12	0	2
" 6 "	36	52	0	2	0	2
" 7 "	42	16	0	1	0	0
" 24 "	0	0	—	—	—	—

It will be seen that there are numerous discrepancies in the different columns—doubtless traceable in part to the loop-method. But it is abundantly clear that one part of peptone per million exerts a marked influence in delaying the death of the staphylococci. This amount of organic matter lies nearly at the limit of detection by chemical means. This biological test is thus at least as delicate as any chemical one for organic contamination of water, and with experiment might prove considerably more delicate.

(2) *Tap water*.—Ordinary tap water (New River Company) was tested on two occasions, on the first with measured 20 c. mm. amounts of the mixture, on the second by the loop method.

TABLE VI.—EFFECT of TAP WATER on STAPHYLOCOCCUS AUREUS at 37° C.

—				Experiment I.	Experiment II.
At start	...	...		213	52
After 1 hour	...	...		—	8
" 2 hours	...	...		98	2
" 3 "	...	...		40	0
" 24 "	..	...		0	—

The effect of tap water is thus seen to be practically identical with that of distilled water; the staphylococci speedily perish at 37° C.

### (3) *Solutions of Sodium Chloride.*

TABLE VII.—EFFECTS of SODIUM CHLORIDE SOLUTIONS of VARIOUS STRENGTHS UPON STAPHYLOCOCCUS AUREUS at 37° C.

Percentage of Na Cl.	0.75 per cent.	0.9 per cent.	0.9 per cent.	1 per cent.	1.5 per cent.
At start...	66	293	64	111	114
After 1 hour	39	152	—	—	—
" 2 hours	5	0	—	4	16
" 3 "	1	0	0	0	2

The effect of normal saline solution and of other solutions of sodium chloride is seen to be identical with that of plain water. A further series of tests was made with Sodium Chloride solutions ranging from 1.5 to 10 per cent.; the results were the same, the cocci perishing by the end of the third hour.

### (4) *Solutions of other Salts and Simple Organic Substances.*

The substances tested comprised potassium nitrate, ammonium tartrate, asparagin, urea, glucose and starch. All were tested in 1 per cent. solution in distilled water.

**TABLE VIII.—EFFECTS of VARIOUS SALTS and SIMPLE ORGANIC BODIES in 1 PER CENT. SOLUTION UPON STAPHYLOCOCCUS AUREUS at 37° C.**

—	Potassium Nitrate.	Ammonium Tartrate.	Asparagin.	Urea.	Glucose.	Starch.
At start ...	119	125	82	121	99	100
After 2 hours ...	0	31	1	29	23	102
" 4 " ...	—	19	0	—	—	—
" 24 " ...	—	0	0	0	0	0

It is seen that 1 per cent. solutions of all these substances exert the same bactericidal action upon the Staphylococcus as has been seen with distilled water.

**(B).—PRELIMINARY EXPERIMENTS with SOLUTIONS CONTAINING PROTEID MATTER or OTHER SUBSTANCE CAPABLE of FURNISHING NUTRIMENT to BACTERIA.**

With the exception of the tests shown in Table V., none of the solutions tested in the experiments hitherto detailed have contained any nitrogenous substance capable of furnishing nourishment to the staphylococcus. No opportunity for multiplication was afforded, and the mortality amongst the cocci steadily progressed up to complete sterility, and this in a few hours. When the solution tested contains a suitable nitrogenous pabulum, the result is a very different one.

**TABLE IX.—EFFECT of PROTEID SOLUTIONS UPON STAPHYLOCOCCUS AUREUS at 37° C.**

—	Peptone 1 per cent.	Peptone 1 per cent. Na Cl 0·5 per cent.	Peptone 1 per cent. Na Cl 0·5 per cent.	Acid muscle extract (Fleischwasser).	Broth.	Broth.	Broth.
At start ...	41	23	101	30	34	92	150
After 1 hour	40	36	—	30	30	86	153
" 2 hours	58	42	135	51	41	101	286
" 3 "	100	98	—	220	68	315	1,372
" 4 "	—	—	227	—	—	—	—
" 24 "	un-countable	—	un-countable	—	—	—	—

In all these experiments there is seen a progressive multiplication of the cocci, already manifest in two hours, and marked in three hours. There is practically no initial drop in numbers.

Even the slight acidity of the beef extract used in preparing ordinary nutrient broth, failed to inhibit the growth of the staphylococcus.

### *Summary of Preceding Experiments.*

It may be claimed, from the data of the foregoing experiments, that the method employed furnishes reasonably consistent and reliable results.

It is shewn that, at a temperature of 37° C., *Staphylococcus pyogenes aureus* rapidly perishes in distilled water and in tap water. It is further shewn that the addition of various salts and organic substances incapable of serving as pabulum for the cocci does not in any way check this mortality, but that the addition of anything more than a trace of proteid material entirely reverses the course of events, permitting not only of survival, but of a multiplication which is already apparent in two hours.

The facts we have set forth thus suggest, though they do not completely prove, that the factor which determines death of the cocci under the conditions named, is absence of pabulum. If it were a question of osmotic or other physical influence, it might have been expected that the various concentrations of sodium chloride tested would have revealed some modification in the result; this was not the case. It may be that the activities of bacterial protoplasm, at 37° C. leave the cocci no choice but multiplication or death: such a conception would explain the facts we have enumerated above.

Our data support the following conclusions:—

- (1) that in water, and in solutions of simple substances incapable of affording bacterial nutriment, the drop in the numbers of cocci present is a rapid and fairly uniform one, with no preliminary multiplication.
- (2) that in artificial proteid media, in which no question as to the presence of specific anti-bodies can arise, there is multiplication almost from the first. No initial drop of any moment is manifest, though the first hour or so is occupied by a phase of repose in which the number of cocci remains fairly constant. There is nothing to suggest that this phase of apparent repose is really the resultant of an initial drop masked by commencing multiplication, though it would be difficult to disprove such an idea.

The actual figures may be summarized as follows:—

- (a) If the number of cocci found at hourly intervals in the five experiments with distilled water given in Table II. be calculated as percentages and reduced to an average, the figures are:—

At the start ...	...	...	...	100.0
After 1 hour	...	...	...	45.8
„ 2 hours	...	...	...	7.8
„ 3 „	...	...	...	0.4

- (b) Similar treatment of the data as to the behaviour of the cocci in artificial proteid media given in Table IX. gives as the average of the seven experiments :—

At the start ...	...	...	...	100.0
After 1 hour	...	...	...	106.2
" 2 hours	...	...	...	149.8
" 3 "	...	...	...	476.7

These figures will serve us later for comparison with the behaviour of cocci in blood.

### C.—EXPERIMENTS CARRIED out by the MORTALITY RATE METHOD on BLOOD.

#### *Defibrinated Blood.*

*Experiment I.*—The result of tests carried out with blood freshly drawn from four normal rabbits and defibrinated is seen in the following tables. Table X. shews the actual figures; Table XI. the same, reduced to percentages.

TABLE X.—EFFECT of NORMAL DEFIBRINATED RABBITS' BLOOD upon STAPHYLOCOCCUS AUREUS at 37° C.

—	Rabbit A.	Rabbit B.	Rabbit C.	Rabbit D.
At start ...	124	105	145	135
After 1 hour ...	75	71	126	75
" 2 hours ...	58	73	101	53
" 4 " ...	126	692	227	274

TABLE XI.—DEGREE of PERCENTAGE REDUCTION EFFECTED.

—	Rabbit A.	Rabbit B.	Rabbit C.	Rabbit D.
At start ...	100	100	100	100
After 1 hour ...	60.5	67.6	86.9	55.5
" 2 hours ...	46.8	69.5	69.6	39.2
" 4 " ...	101.6	659.0	156.5	202.9

It is clear from these results that the defibrinated blood of the normal rabbit has not merely an inhibitory but a distinctly bactericidal effect upon the staphylococcus during the first few hours of contact. After four hours, however, the coccus has begun to multiply.

A number of further experiments made with normal rabbits' blood have confirmed this result. In all instances there was a decrease in the numbers of the staphylococcus during the first two hours, and in some cases it was not until the fifth hour that the coccus began to multiply.

*Experiment II.*—In this the defibrinated blood of a normal rabbit was compared with that of an immune rabbit, while ordinary broth was used as a control. At the same time samples of the immune rabbit's blood were tested which had been saturated with carbon dioxide and carbon monoxide respectively, in the one case by passing expired air, and in the other coal gas, through it for five minutes.

The immunisation of the rabbit in question had been carried out as follows: on July 8th, it had received, intravenously, 1 mgm. of the coccus, killed by heat: on July 15th it received 2 mgm. and on July 22nd 4 mgm. of killed cocci, also intravenously. The test was carried out on July 30th; the degree of immunity was probably not very great.

TABLE XII.—CONTRASTING the EFFECT of NORMAL and IMMUNE RABBITS' BLOOD DEFIBRINATED, with BROTH as a CONTROL—also the EFFECT of SATURATING the IMMUNE BLOOD with CO<sub>2</sub> and CO.

	Defibrinated blood of normal rabbit.	Defibrinated blood of. immune rabbit.	Defibrinated blood of. immune rabbit saturated with CO <sub>2</sub> .	Defibrinated blood of immune rabbit saturated with CO.	Ordinary broth as control.
At start ...	153	163	156	166	150
After 1 hour	22	30	95	127	153
" 2 hours	18	9	99	75	286
" 3 "	13	17	78	97	1,372
" 4 "	139	18	96	161	(approx.) uncount- able.
" 5 "	776 (approx.)	84	1,296 (approx.)	856 (approx.)	"
" 6 "	uncount- able.	1,388 (approx.)	uncount- able.	uncount- able.	"

In this experiment the destructive action of the blood upon the coccus is very noteworthy. The normal rabbits' blood produced a 91 per cent. reduction by the end of the third hour, and not till after the end of the fourth hour did the number of cocci again reach the initial number. The blood of the immune animal produced a 94 per cent. reduction by the end of the second hour, and an inhibitory effect is manifested during the third and fourth hours; not till after the end of the fifth hour did the cocci again reach their initial number. The action of the immune rabbits' blood is thus perceptibly more energetic and enduring than that of the normal animal. Saturation with carbon dioxide and monoxide seemed in this experiment definitely to reduce the anti-bacterial action of the immune blood, but we are at present unable to furnish an explanation of this; we have not as yet repeated the observation. The control experiment with broth supplies an effective contrast; multiplication is uncontrolled from the start.

The foregoing experiments indicate the presence in normal rabbits' blood, and still more in immune blood, of some bactericidal substance which is of considerable activity during the first few hours of contact; but the action of which becomes exhausted by the end of the 3rd or 4th hour.

*Experiment III.*—The effect of heat upon this bactericidal substance was next tested. Blood was withdrawn from two normal rabbits and defibrinated; each sample was divided into two parts, one of which was exposed for 30 minutes in a water bath to a temperature of 55°–56° C. The result of the tests was as under:—

TABLE XIII.—EFFECT of a TEMPERATURE of 55° C. upon the BACTERICIDAL POWER of DEFIBRINATED BLOOD from TWO NORMAL RABBITS.

	Defibrinated blood of Rabbit I.		Defibrinated blood of Rabbit II.	
	Unheated.	Heated.	Unheated.	Heated.
At start ... ..	157	161	91	74
After 2 hours ... ..	25	139	30	78
After 4 hours ... ..	7	136	20	69
After 5 hours ... ..	14	113	—	—

It appears from these results that previous exposure for half-an-hour to a temperature of 55°–66° C. very greatly diminishes the destructive effect of the blood upon the staphylococcus; the bactericidal substance is largely thermo-labile.

*Experiment IV.*—Inasmuch as the preliminary observations carried out with water, saline solutions, &c., pointed to lack of pabulum as the explanation of the rapid disappearance of the staphylococcus, we thought it right to exclude this as a possible cause of their early diminution in normal blood, although their

TABLE XIV.—EXPERIMENTS to show that the INITIAL MORTALITY seen in COCCI EXPOSED to DEFIBRINATED BLOOD is not due to lack of PABULUM.

	Normal rabbits' blood defibrinated and unheated.	The same, with the addition of 1/100 of its volume of previously heated blood.	The same, with the addition of 1/20 of its volume of previously heated blood.	The heated blood alone. (55° C. for 30 min.)
At the start ... ..	91	89	75	74
After 2 hours ... ..	30	45	36	78
" 4 " ... ..	20	20	11	69

TABLE XIV.—*continued.*

—				Fresh Normal defibrinated rabbits' blood.	The same, with the addition of 1/20 of its volume of 10 per cent. peptone solution.
At the start ...	...	...	...	146	146
After 2 hours	...	...	...	59	65
" 4 "	...	...	...	58	58

rapid multiplication after the first few hours renders such a suggestion highly improbable. Since the heated blood scarcely impedes their growth, the effect of adding small amounts of heated to the unheated blood was tried: also the effect of adding peptone.

It is seen from these experiments that the addition of adventitious pabulum has no effect in hindering the bactericidal action of the unheated blood.

*Experiment V.*—The bactericidal substance would be brought into line with what is known of other anti-bodies (bactericidal and other) could it be shown to become anchored to the cocci. The following experiments were carried out to see whether saturation of the defibrinated blood with cocci, killed by heat, deprived it of its bactericidal power upon living cocci. A suspension of *Staphylococcus aureus* was killed by exposure to 65°C. for 30 minutes, and its sterility proved by subculture. One cc. of fresh defibrinated rabbits' blood was placed in each of two tubes, and to one of the two was added 1/25 cc. of the killed coccus emulsion. The two tubes were kept side by side in an incubator at 37°C. in the first experiment for one, in the second for two hours. They were then inoculated according to the ordinary routine with living cocci and tested.

TABLE XV.—The EFFECT of SATURATING DEFIBRINATED RABBITS' BLOOD with KILLED COCCI.

—		Rabbit 1 (immune).		Rabbit 2 (normal).	
		Defibrinated blood untreated.	The same, treated with killed suspension of staphylococci for 1 hour.	Defibrinated blood untreated.	The same, treated with killed suspension of staphylococci for 2 hours.
At start ...	...	240	287	160	187
After 2 hours	...	118	215	73	279
" 4 "	...	100	234	77	uncountable.

It is clear from these figures that the preliminary saturation with killed cocci so far "anchored" the bactericidal substance as to remove, in the first case the greater part, and in the second case apparently the whole of it. The difference is evidently traceable to the fact that in the first case the preliminary saturation was carried on for an insufficient time (one hour), whereas in the second case contact was allowed for two hours. In another experiment with a normal rabbit in which only one hour's preliminary saturation was allowed, there was little difference between the saturated and the untreated serum.

(b) Serum.

Having by the above experiments satisfied ourselves of the existence of a substance bactericidal to *Staphylococcus pyogenes aureus* in the defibrinated blood of the rabbit, further investigations were carried out upon the serum alone.

*Experiment VI.*—In this the effects of serum from three normal rabbits was tested.

TABLE XVI.—The EFFECT of NORMAL RABBITS' SERUM upon STAPHYLOCOCCUS AUREUS at 37° C.

—	Rabbit 1.	Rabbit 2,	Rabbit 3.
At start ... ..	79	204	179
After 1 hour ... ..	21	—	—
„ 2 hours ... ..	24	165	26
„ 3 „ ... ..	9	—	—
„ 4 „ ... ..	—	440	8
„ 5 „ ... ..	—	uncountable	14

It is plain that, during the first few hours of contact, the serum of a normal rabbit exerts a destructive effect upon the cocci similar to that seen in the case of defibrinated blood. It would also appear that different rabbits may present striking individual variations in the amount of bactericidal substance present in the serum. There is a marked contrast in the above experiment between the sera of rabbits 2 and 3.

*Experiment VII.*—Serum from three rabbits was heated to 55°–56° C. for half an hour and contrasted with unheated serum from the same animals. One of the rabbits was normal, the remaining two were in process of immunisation against the staphylococcus, having already received doses of killed cocci intravenously; they may be regarded as partially immune.

TABLE XVII.—The EFFECT of HEATING NORMAL and PARTIALLY IMMUNE RABBITS' SERUM.

	Normal rabbit.		Partially immune rabbit A.		Partially immune rabbit B.	
	Unheated serum.	Heated serum.	Unheated serum.	Heated serum.	Unheated serum.	Heated serum.
At start ...	192	160	177	169	162	189
After 2 hours	73	144	45	137	34	148
" 4 " ...	31	184	15	190	12	103
" 5 " ...	40	153	15	493	11	115

The figures show that, just as in the case of defibrinated blood, heating to 55°–56° C. for half an hour largely does away with the destructive action of the serum upon the cocci.

They also indicate that a somewhat higher degree of bactericidal power is present even in the partially immunised animal, as compared with the normal. Thus the maximum effect of the unheated serum of the normal animal in the above experiment was an 84 per cent. reduction, whereas with the partially immunised animals it amounted respectively to 91 and 93 per cent.; further, the cocci began again to increase in the normal earlier than in the partially immune serum (unheated).

TABLE XVIII.—The EFFECT of SATURATING NORMAL RABBITS' SERUM with KILLED STAPHYLOCOCCI.

—		Serum.	Saturated serum.
At start ...	...	186	156
After 1 hour	...	129	153
" 2 hours	...	77	174
" 3 "	...	22	162
" 3½ "	...	13	164

Here, also, as in the case of defibrinated blood, the killed cocci removed the destructive substance from the serum.

(c) *Experiments with Human Blood.*

These experiments were carried out with blood from a venesection, which was performed on a man brought to St. Bartholomew's Hospital in the early stages of a cerebral hæmorrhage. We have to thank Dr. H. Morley Fletcher for his kindness in acquainting us with the fact that the bleeding was to be performed. The blood was defibrinated and was in part tested as such; serum was also obtained from it and tested in various ways. Further, some of the serum was saturated with a suspension in normal

saline of staphylococci killed by heat and proved sterile by culture (40 c.mm. of the suspension to 1 cc. serum—allowed to remain in contact all night on ice); this was tested on the following day. The results of these experiments are seen in the following tables:—

TABLE XIX.—The EFFECT of HUMAN DEFIBRINATED BLOOD and SERUM, UNHEATED and HEATED, on STAPHYLOCOCCUS AUREUS at 37° C.

—	Defibrinated blood unheated.	Defibrinated blood after heating to 55° C.	Serum unheated.	Serum after heating to 55° C.
At start ...	142	166	127	142
After 2 hours ...	103	124	102	138
" 4 " ...	75	100	76	100

or, EXPRESSED as PERCENTAGES,

—	Serum alone unheated.	Serum saturated with killed cocci.	Serum heated to 55° C. for 45 mins.	Serum heated and saturated with cocci.
At start ...	100	100	100	100
After 2 hours ...	72	74	80	97
" 4 " ...	52	60	59	70

TABLE XX.—The EFFECT of HUMAN SERUM, kept for 24 HOURS on ICE (UNHEATED, HEATED, and SATURATED with KILLED STAPHYLOCOCCI) on STAPHYLOCOCCUS AUREUS at 37° C.

—	Serum alone unheated.	Serum saturated with killed cocci.	Serum heated to 55° C. for 45 mins.	Serum heated and saturated with cocci.
At start ...	202	207	391	214
After 2 hours ...	122	209	261	235
" 4½ " ...	Uncountable	Uncountable	Uncountable	Uncountable

or, EXPRESSED as PERCENTAGES,

—	Serum alone unheated.	Serum saturated with killed cocci.	Serum heated to 55° C. for 45 mins.	Serum heated and saturated with cocci.
At start ...	100	100	100	100
After 2 hours ...	60	100	66	109
" 4½ " ...	α	α	α	α

It is apparent that these experiments, so far as they go, confirm the results obtained with rabbit's blood. There is a manifest reduction of cocci in contact with the unheated blood or serum during the first few hours; and this action is to some extent hindered by previous heating to 55° C., and by saturation with killed cocci; still more by both combined.

*(d) Experiments with Human Urine.*

Two sterilised urines were tested, one a very acid, the other a slightly acid, specimen.

TABLE XXI.—THE EFFECT of HUMAN URINE upon STAPHYLOCOCCUS AUREUS at 37° C.

—				Urine 1 (very acid).	Urine 2 (slightly acid).
At start	...	...	...	207	198
" 2 hours	...	...	...	108	183
" 8 "	...	...	...	118	346
" 24 "	...	...	...	Uncountable	Uncountable

In these experiments there is seen a marked initial drop in the number of the cocci in the acid specimen of urine and a slight drop in the slightly acid specimen. In the latter the cocci had already begun to multiply in three hours, and in both there was enormous increase in 24 hours, by which time both urines were alkaline in reaction. The experiment with acid muscle extract, included in Table IX., shows that mere acidity cannot be the only determining factor. It is possible that human urine normally contains some of the anti-body which destroys the cocci, *i.e.*, that the bactericidal substance normally present in the blood is in part excreted in the urine.

*General Conclusion from the Preceding Experiments upon the Blood.*

The experiments above detailed appear to show, not merely an inhibitory, but some degree of bactericidal, action upon *Staphylococcus pyogenes aureus*, on the part of defibrinated blood and serum, both from the rabbit and from man.

*Criticism.*—But before accepting this conclusion it is necessary to consider in some detail whether this bactericidal action is real or apparent only. The method we have used is open to the criticism that it measures results by the number of colonies which arise in the final plate cultures. May not the decrease in the number of colonies, under the influence of blood or serum, be due to agglutination of the cocci?

If an emulsion of staphylococci be made from a young agar culture in normal saline solution, and equal drops of this and of rabbit serum be mixed on a slide and incubated at 37° C., microscopic examination shows a greater or lesser degree of clumping amongst the cocci within an hour, or even in ten minutes. The serum of an immunised rabbit may cause "complete" agglutination in an hour under the conditions named (equal parts of serum and bacterial emulsion), *i.e.*, all the cocci are collected into large clumps, and practically no free individuals are seen. If these were the conditions present in the mixtures of defibrinated blood or serum with staphylococcal emulsion, it is clear that the fallacy

introduced by the clumping might well render the method worthless for numerical estimation of the cocci present. But the conditions under which we have worked are widely different.

An ordinary broth culture of the staphylococcus, 24 hours old, may contain, on an average, 2,000 million cocci per cc.; mixed with an equal volume of serum this number would be halved. But in the mixtures with which we have worked, the cocci number from 2,000 to 2,500 per cc., or some 500,000 fold less than in the mixture usually employed in agglutination tests. The actual distance between the individual cocci, supposing these to be uniformly distributed, will be  $\sqrt[3]{500,000}$ —roughly 80 times greater in the one case than in the other. We do not understand the precise mechanism of agglutination, but it may fairly be assumed to be a physical process obeying physical laws, amongst others that which governs the action of attractive forces according to the inverse square of the distance. If this were so, agglutinating action should be some 6,400 fold less active in our mixtures than in an ordinary agglutination test. This is not to say that it may not occur, and even to some extent influence the results obtained, but it renders it improbable that it plays a determining share in the results, indeed we are disposed to think it almost negligible in its influence.

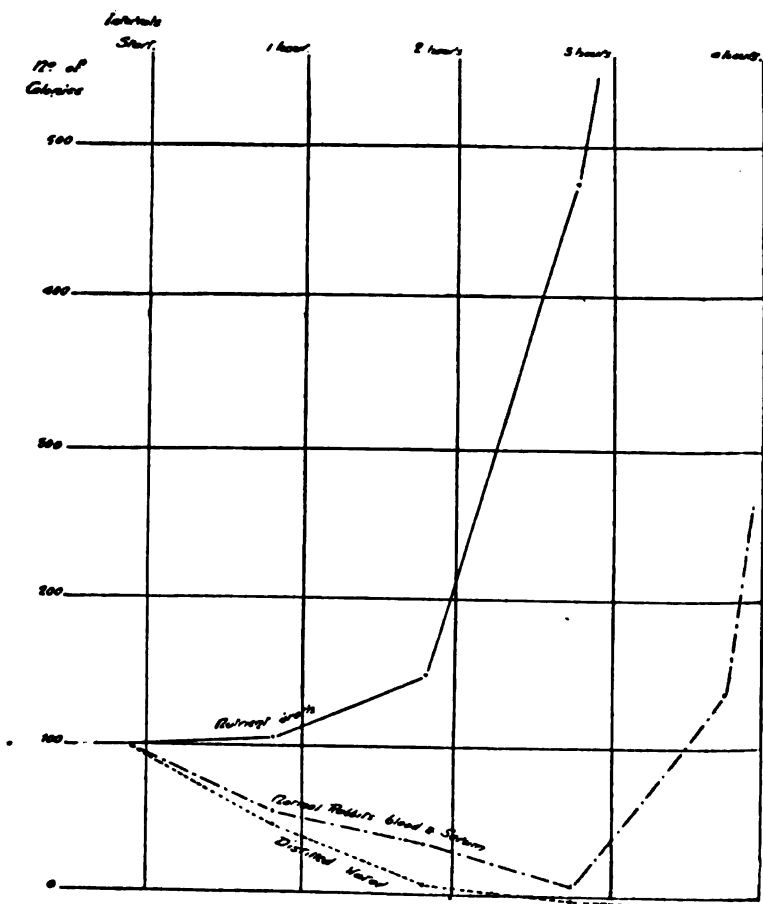
Further, a consideration of the curves obtained by plotting out our results graphically will show that they could not readily be explained merely on the theory of agglutination. There is seen a steady fall in the apparent number of cocci present up to the end of the third or fourth hour, the reduction being from 50 to 95 per cent. of the original total. Then an abrupt rise occurs, which on the hypothesis of agglutination, could only be explained by a sudden disruption of the clumps, for which no ocular evidence is forthcoming in agglutination experiments. In those agglutination experiments with staphylococci which we have followed under the microscope, clumping has practically attained its maximum by the end of the first hour.

*Conclusions.*—For these reasons, therefore, we consider our results best explained on the hypothesis that there exists in the blood a substance which actually destroys the staphylococci and not merely agglutinates them or otherwise inhibits their activities. It is thermo-labile, and can be "anchored" to the cocci, and can thus readily be removed from the blood. It is, however, clearly of a different order, at least quantitatively, from that which normal or immune blood possesses against such bacteria as the cholera vibrio or the typhoid bacillus. For in none of our experiments has the destructive action of the blood or serum upon the cocci been complete; by the fourth or fifth hour the action appears exhausted, and the cocci then proceed rapidly to multiply, as in any other nutrient medium; the initial drop in numbers escapes detection unless some such method be employed as we have described.

The results which we have obtained by this method may conveniently be expressed in the form of a diagram, contrasting the influence upon the staphylococcus of (1) distilled water, the effect of which is purely destructive, (2) artificial nutrient media, such

as broth, in which no "antibody" can possibly be present, and (3) normal rabbit's blood and serum in which such antibodies are indubitably present, if only to small extent. In each case the curve is calculated from the mean of several experiments.

DIAGRAM II.—CURVES shewing the EFFECT of the first few HOURS' CONTACT with STAPHYLOCOCCUS AUREUS, of (1) DISTILLED WATER, (2) NUTRIENT BROTH, and (3) NORMAL RABBITS' BLOOD and SERUM, at  $37^{\circ}$  C. in terms of the percentage NUMBER of COLONIES which arise.



These curves summarise the results we have obtained by the application of the "mortality rate" method to *Staphylococcus aureus*. In nutrient broth, where no adverse condition is present, the rise in the number of cocci is seen to be unchecked. In distilled water, where from absence of pabulum the conditions

are wholly adverse, the fall in numbers is unchecked and zero is reached in three or four hours. In normal rabbits' blood, defibrinated, or in serum, there is seen to be an initial fall in numbers, almost comparable with that seen in distilled water; but during the fourth hour checked and replaced by a rise similar to that seen from the first in nutrient broth. We have given our reasons for believing that this initial drop is not merely an apparent one due to agglutination; though we cannot exclude this as taking some share in the phenomenon. It is not due to deficiency of pabulum, and it cannot be due to opsonic action; for it occurs equally in absence of leucocytes. It is therefore, presumably, in the main, a directly bactericidal action.

If this conclusion be accepted, the demonstration is not unimportant. Even though the destruction of 100 or 150 cocci by 1 cc. of serum *in vitro* is incomplete, even though the destructive action is exhausted at the end of three or four hours, the reaction may be of great value in bodily defence. For the process of infection, as it naturally occurs, must usually consist in invasion of the tissues, not by battalions, but by units which later multiply into battalions. A bactericidal power, even though of relatively slight degree, may suffice to annihilate the first units, and a reduction of 50 to 95 per cent. in numbers is more than a slight means of defence. It may be a valuable adjunct, or rather preliminary, to the more active defence possessed by the body in its phagocytes.

### SECTION III.

#### PRELIMINARY OBSERVATIONS ON THE COURSE OF INFECTION WITH STAPHYLOCOCCUS PYOGENES AUREUS IN THE LIVING RABBIT, AND ON THE NATURE OF THE DEFENSIVE MECHANISMS BROUGHT INTO PLAY.

The value of experiments, carried on outside the body, as to the defensive mechanisms possessed by the living organism against bacterial invasion, is beyond dispute; and such experiments form an essential preliminary to the investigation of the sequence of events *intra vitam* when infection takes place. While, therefore, we have devoted most of our time to testing the means which may be employed for measuring the defensive mechanisms *in vitro*, we have throughout regarded this work as preliminary to the real questions at issue. The processes of infection and defence must, in the ultimate event, be judged by what is observed in the living body of the infected animal, or *in vivo*.

We have commenced such observations, and in this section of our report we record investigations carried out, in more or less detail, upon seven rabbits submitted to intravenous inoculation with known doses of *Staphylococcus pyogenes aureus*. In these experiments we have noted the content of the circulating blood in staphylococci at various times after inoculation—up to recovery or death, and for the technique of the method we have employed, as well as for help in the work, we are much indebted to Dr. T. J. Horder. We have also paid special attention to the number of

leucocytes in the circulating blood, and have commenced an investigation of the leucoblastic reaction of the bone-marrow after death. The opsonic value of the serum has also in certain cases been determined.

#### GENERAL REMARKS ON STAPHYLOCOCCUS INFECTION IN THE RABBIT.

The rabbit possesses a moderate degree of natural immunity against staphylococci—a degree which may fairly be compared with that of man. It was this fact which influenced us in selecting this animal as the subject of our observations. Subcutaneous inoculation of the rabbit with *Staphylococcus aureus*, in suitable dose, leads to a local suppuration from which the animal usually recovers. It is distinctly less susceptible than the guinea-pig to general infection, when the coccus is inoculated subcutaneously. When, however, the coccus is introduced into the circulation in suitable dose, by direct intravenous injection, death occurs after one to ten days; not as a rule by a fulminant septicæmia, for unless the dose is overwhelming, the cocci soon disappear temporarily or permanently from the blood; but by a more or less localised “pyæmia” in which metastatic foci of suppuration in certain organs, notably the kidneys and less commonly the heart muscle, play a predominant part. The extent of the visible pyæmic changes depends chiefly on the length of time which elapses before the animal succumbs. The fatal dose of living *Staphylococcus pyogenes aureus*, administered intravenously to a normal adult rabbit, must vary with the virulence of the strain of coccus employed, and with the individual resistance of the animal. But in general terms our experience has been that  $\frac{1}{4}$  a milligramme of a 24-hour culture on agar proves fatal in four or five days. At times, such a dose may be fatal in 24 hours; at other times death may be delayed to the tenth day. Half a milligramme of culture corresponds roughly to 250 million cocci. It must carefully be borne in mind that any clinical parallel to this dose of cocci must be of extremely rare occurrence in such infections as are seen naturally in man. Sudden irruption into the circulation of such an enormous dose of the microbe, could only be brought about by the rupture of an abscess into a blood vessel of some size, and here the patient would already be protected in some degree by the formation of anti-bodies, during the gradual maturation of the focus of suppuration. The usual primary mode of infection in man must be by the invasion of the tissues and not of the blood stream, and by a limited number of cocci in the first instance.

We have not been unmindful of this lack of quantitative correspondence between human infections and the mode of experimentation which we have adopted. But we have, nevertheless, employed large intravenous doses in the rabbit as affording us the opportunity of studying the means of defence brought into play where the infection is a sudden and dangerous one, running its course in a few days. Moreover, it must not be forgotten that increased virulence of the coccus, or diminished resistance thereto, will tend to equalise this divergency due to difference of dose.

The dead bodies of *Staphylococcus aureus* can be introduced into the circulation of the rabbit in enormous numbers without harmful result. One, two, or five milligrammes of dead culture are well borne, and in one case a cumulative dose aggregating 30 milligrammes was injected at half hourly intervals without causing death.

In the experiments to be recorded, we have looked upon each animal inoculated as a "clinical case," to be studied from as many points of view as possible.

**CASE I.**—A rabbit (numbered N. in our note book), weighing 2,040 grammes, was inoculated intravenously with an amount of bacterial emulsion corresponding to half an agar culture of *Staphylococcus aureus*. Before inoculation, the circulating leucocytes had numbered 1,600 per c.mm. of blood.

*Two hours* after inoculation the number of leucocytes had risen to 2,560 per c.mm. 1/10 cc. of blood withdrawn from a vein at this stage yielded 34 colonies of coccus, and 1/100 cc. yielded 13 colonies.

*Six and half hours* after inoculation the leucocytes numbered 2,400 per c.mm. 1/10 cc. of blood yielded over 100 colonies of staphylococci, while two cultures, each made with 1/100 cc. of blood, yielded respectively 27 and 31 colonies.

On the following morning the animal was found dead. The body weighed 2,010 grammes—having thus lost 30 grammes in weight in less than 24 hours.

*Remarks.*—In this experiment a dose of cocci was employed greatly in excess of the minimum lethal dose, and much larger than any we used in subsequent experiments. The observations shew a progressive increase in the number of cocci circulating in the blood—a true staphylococcal septicæmia, to which the animal succumbed in less than 24 hours, with practically no response in the way of leucocytosis. The rapid loss of weight in a short time is noteworthy.

**CASE II.**—A rabbit weighing 1,940 grammes (Aureus No. 5 in our note book) was inoculated with 0.5 milligramme of a recent agar culture of *Staphylococcus aureus* into the ear vein. The leucocytes at the time of inoculation numbered 2,200 per c.mm., and the opsonic index, as compared with an uninoculated rabbit used throughout the whole experiment as a control, was 0.5. (In estimating the opsonic indices all cocci over 10 per leucocyte were disregarded.) The animal died on the fourth day. The observations made on it are seen in the following table and diagram.

From the diagram the course of events is better appreciated than from the Table. The following points are worthy of notice.

(1.) The coccus rapidly diminished in numbers in the circulation, and in four hours could no longer be detected in 1/10 cc. of blood. It reappeared in small and fluctuating numbers about the seventh hour after inoculation, but the degree of septicæmia was trivial for 48 hours, when the number of circulating cocci began

to undergo a great and progressive increase which was maintained up to death.

(2.) The degree of leucocytosis was in this case slight; during the first few hours counts of 11,000 and 9,000 leucocytes per c.mm. were the highest observed, and these were but temporary efforts.

(3.) The opsonic index shows a distinct rise, with a fall before death. Its curve shows some parallel with that of the leucocytosis.

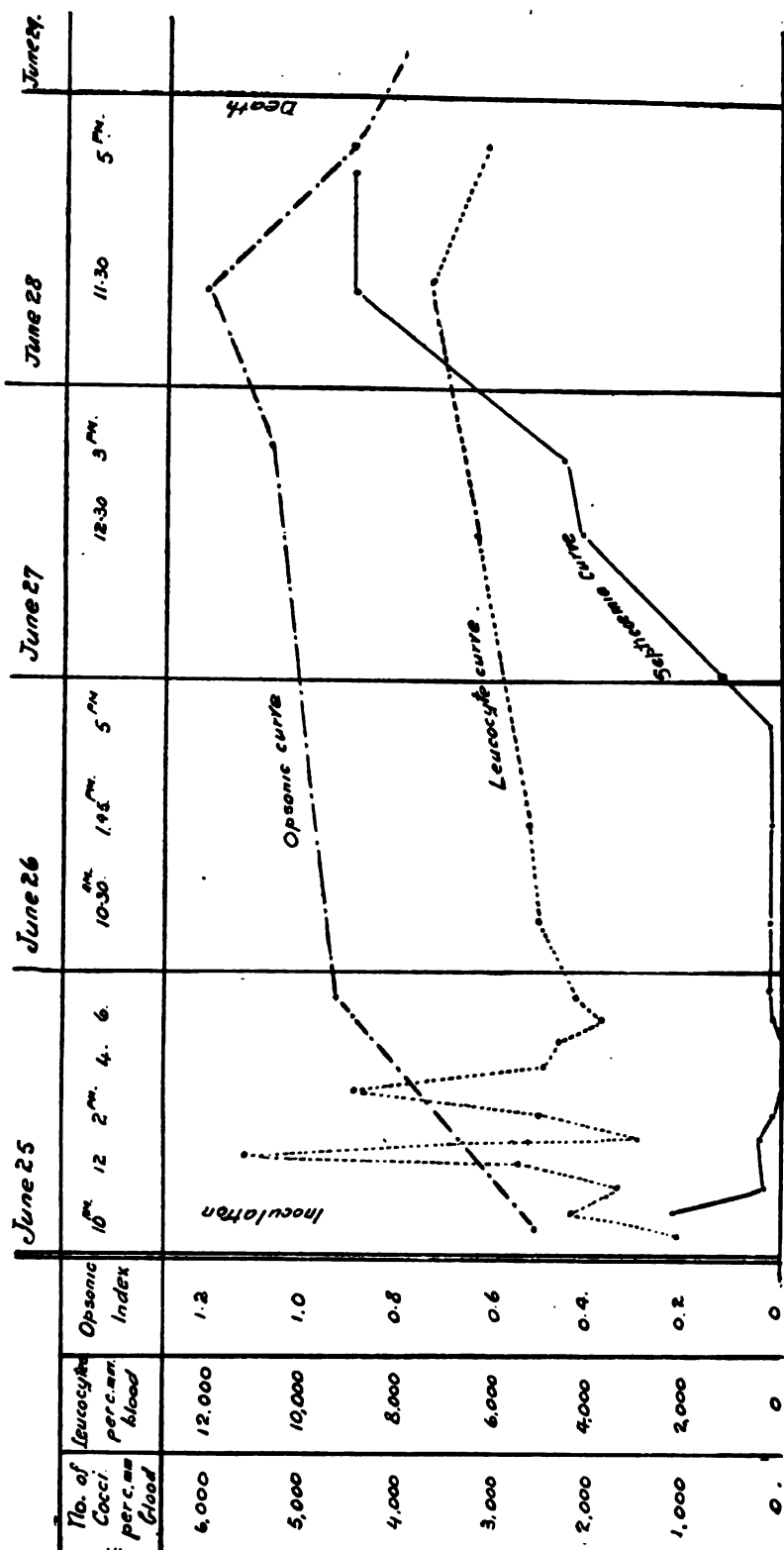
(4.) The great loss of weight which occurred during the illness took place entirely during the last two days of life, when resistance was exhausted and septicæmia was pronounced.

TABLE XXII.—SHOWING the DEGREES of SEPTICÆMIA and LEUCOCYTOSIS and the VARIATIONS in the OPSONIC INDEX in a NORMAL RABBIT which DIED on the FOURTH DAY after INTRAVENOUS INOCULATION with 0·5 mgm. of *STAPHYLOCOCCUS AUREUS*.

Date.	No. of cocci in 1/10 cc. blood.	No. of cocci in 1/100 cc. blood.	No. of cocci in 1/1000 cc. blood.	No. of leucocytes per c.mm. blood.	Opsonic index.	Weight in grammes.
June 25—						
10 a.m. Inoculation of 5 mgm.				2,200	0·5	1,940
10.30 a.m. ...	Numerous	12	0	4,400		
11 a.m. ...	Several	2	4	3,400		
12 midday ...	5	1	1	5,600		
12.30 p.m. ...				11,200		
1 p.m. ...	Several	Several	1	3,000		
2 p.m. ...	Several	0	0	5,000		
3 p.m. ...	0	0	0	9,000		
4 p.m. ...	0	0	0	5,000		
5 p.m. ...	0	0	0	4,600		
6 p.m. ...	2	1	0	3,800		
7 p.m. ...	0	1	0	4,200	0·93	
June 26—						
10.30 a.m. ...	Several	0	2	5,000		1,950
1.45 p.m. ...	0	1	0	5,200		
5 p.m. ...	1	1	1			
June 27—						
12.30 p.m. ...	Countless	21	2	6,400		1,800
3 p.m. ...	Countless	23	5		1·07	
June 28—						
10.30 a.m. ...	Countless	40-50	4	7,400	1·2	1,680
5 p.m. ...	Countless	Many	4	6,200	0·9	
June 29—						
Dead ...					0·8 post mortem.	

DIAGRAM III.

SHOWING the COURSE of the SEPTICÆMIA and the DEGREE of LEUCOCYTOSIS together with the OPSONIC CURVE in the above instance of FATAL INFECTION with STAPHYLOCOCCUS AUREUS.



*General Remarks on Case II.*

The experiment well illustrates the sequence of events when a fatal, but not excessive, dose of staphylococci is suddenly introduced into the circulation of an animal not specially protected. The temporary disappearance of the coccus from the blood after a few hours will be seen, in later experiments, to be a usual thing in this form of infection. The bactericidal influence of the serum to which we have drawn attention may be partially concerned in this; doubtless some degree of phagocytosis also occurs, though to what extent this takes place in the circulating blood we have no evidence. It must, however, be noted that the disappearance of organisms from the blood stream does not necessarily imply a corresponding destruction of cocci. There is reason for suspecting that some of the cocci are "held up," presumably by a process of septic embolism, in certain tissues and organs of the body, and especially in the kidneys. In such regions they may continue to multiply, and may set up fatal mischief with a subsequent secondary re-invasion of the blood. A prominent feature in the failure of the defensive mechanism in this rabbit is seen in the absence of any effective degree of leucocytosis. The opsonic power showed a marked increase; but this cannot be taken as a measure of effective resistance *in the absence of leucocytic response*. Indeed, the opsonic index afforded no clue whatever to the gravity of the animal's condition; the creature was doomed from the outset.

CASE III.—In this case a rabbit, weighing 2,330 grammes (numbered Rabbit 0 in our case book), was subjected in the course of four months to four successive intravenous inoculations with living *Staphylococcus pyogenes aureus*. It succumbed to the last inoculation. It will be convenient to consider first the sequence or events in the two first infections from which recovery took place.

TABLE XXIII.—SHOWING the RESULTS of TWO INTRAVENOUS INOCULATIONS of a RABBIT with STAPHYLOCOCCUS PYOGENES AUREUS: RECOVERY in each CASE.

Date.	Dose of cocci inoculated.	No. of cocci in 1/10 cc. blood,	No. of cocci in 1/100 cc. blood.	No. of cocci in 1/1000cc. blood.	No. of leucocytes per c.mm. blood.	Weight in grammes.
Feb. 14 (before inoculation)— 2 p.m. ... ..	1st infection, 0.1 mgm. living cocci.				1,600	2,330
6 p.m. ... ..			33		800	2,330
Feb. 15— 11 a.m. ... ..			62			2,480
6.30 p.m. ... ..					4,000	2,470
Feb. 16 ... ..			0			2,410

TABLE XXIII.—*continued.*

Date.	Dose of cocci inoculated.	No. of cocci in 1/10 cc. blood.	No. of cocci in 1/100 cc. blood.	No. of cocci in 1/1000 cc. blood.	No. of leucocytes per c.mm. blood.	Weight in grammes.
May 24— 5 p.m. ...	2nd infection, 1.0 mgm. living cocci.					2,470
7.15 p.m. ...		Several	Several	1 in 1/500 cc.		
May 25— 12.30 p.m. ...		0	0	0	3,200	2,430
May 26— 10 a.m. ....		0	0		10,400	2,400
May 29 ...					6,800	2,170
May 31 ...					2,400	2,060

The following facts are brought out in the preceding table :—

(1.) The first infection was with a small dose of cocci, and although the animal was unprotected by any previous inoculations, recovery took place in a day or two. That no serious illness was produced is manifest from the records of the animal's weight, which, as Dr. Horder pointed out to us, forms a most sensitive index of constitutional disturbance. All cocci disappeared from the blood during the second day, and only a trifling leucocytosis was evoked in response to the infection. The figures illustrate the sequence of events when a sub-fatal dose of staphylococci is introduced into the circulation of a rabbit, and the natural defensive mechanisms of the body are adequate to deal promptly with the invasion.

(2.) The second infection was with a large dose of cocci, double that which is usually fatal to an unprotected animal. But the rabbit was no longer unprotected; the infection from which it had recovered three months previously must have evoked a considerable increase in its capacity for defence, persisting up to the date of the second infection. To this the animal doubtless owed its recovery on the second occasion, yet the table shows evidence of a much more severe illness. It is true that the cocci all vanished from the blood within 24 hours, but the loss of over 400 grammes in weight in a week shows clearly that the constitutional affection was of some severity. The degree of leucocytosis, again, though by no means high, is much higher than with the first infection, and it is noteworthy that it attained its maximum more than 24 hours after the disappearance of the cocci from the circulation, and that it was not till a week after the inoculation that the number of leucocytes became again normal. This would suggest the existence of deep-seated metastatic foci of infection, probably in the kidneys, which were not overcome for several days. The figures in this second infection thus illustrate the course of events when recovery takes place, after a struggle,

in a partially protected rabbit exposed to a dangerous degree of intravascular infection. The third and fourth infections of this rabbit may be considered together because there can be no doubt that the animal had not recovered from the third when the fourth dose of cocci was administered. Each of these doses was but a half of that given on the second occasion, yet their cumulative action led to a fatal issue.

TABLE XXIV.—SHOWING the RESULTS of TWO SUCCESSIVE INTRAVENOUS DOSES of STAPHYLOCOCCUS AUREUS, at an INTERVAL of EIGHT DAYS, upon the PARTIALLY IMMUNE RABBIT whose PREVIOUS HISTORY is RECORDED in TABLE XXIII.

Date.	Dose of cocci inoculated.	No. of cocci in 1/10 cc. blood.	No. of cocci in 1/100 cc. blood.	No. of cocci in 1/1000cc. blood.	No. of leucocytes per c.mm. blood.	Opsonic index.	Weight in grammes.
June 11 ...	3rd infection, 0.5 mgm. living cocci.						
June 13 ...		0	0	0		1.56	
June 15 ...					16,800	1.9	1,530
June 18 ...							1,460
June 19 (before inoculation)— 9.40 a.m. ...	4th infection, 0.5 mgm. living cocci.				2,400		1,460
12.0 ...		Several	2	2 in 1/500 cc.	24,400		
2 p.m. ...		0	0	0	22,000		
4 p.m. ...		0	0	0	29,200		
7 p.m. ...		1	0	0	51,200		1,540
June 20— 11 a.m. ...		0	0	0	28,600		
4 p.m. ...					13,200		1,470
June 21 ...					12,800		1,670
June 22 ...					15,200		1,750
June 23 ...					22,000		
June 24 ...	(Animal moribund) Both kidneys shewed extensive foci of suppuration.				25,200	2.0 (post mortem)	

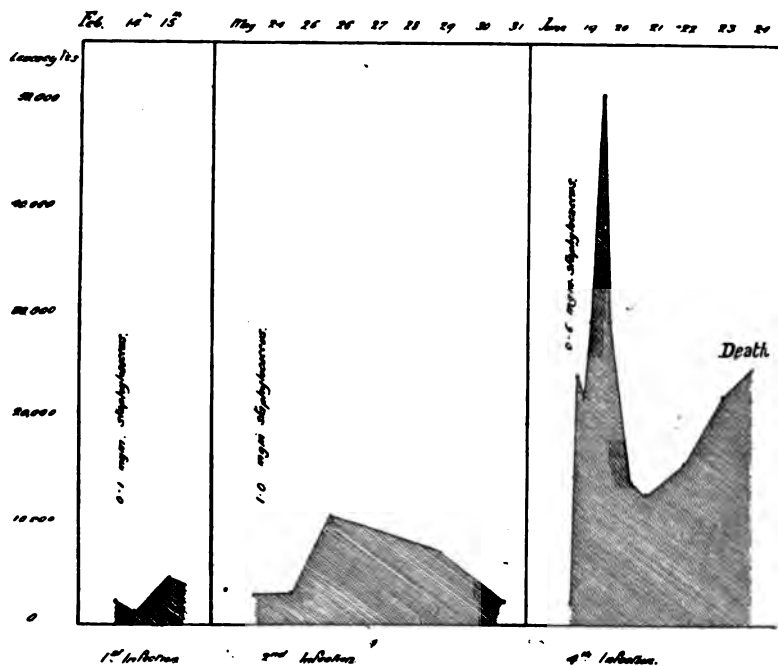
The conclusions which we draw from these figures are as follows :—

1. The data as to the third infection are very incomplete, but all cocci had disappeared from the blood within 48 hours of the infection. Nevertheless it is highly probable that metastatic infection still persisted even on the seventh day; evidence for this is seen in the moderately high leucocytosis seen on this day, and in the considerable loss of weight which was going on.

2. It is hence probable that the fourth intravenous inoculation was one superadded to a deeply seated focus of infection from which the animal had not yet recovered. If so, the condition was one of exceptional gravity, and it is not surprising that the issue was a fatal one. But in spite of this it is seen that the defensive mechanisms of the body, doubtless largely reinforced as a result of the preceding infections, were able to offer a vigorous resistance, death being delayed for five days.

3. The most striking feature of this fourth infection was the promptness and the high degree of the leucocytic response. In the following diagram the degree of leucocytosis from day to day is exhibited for the first, second, and fourth infections of this rabbit; no sufficient data exist as to the third infection.

DIAGRAM IV.—SHEWING the PROGRESSIVE INCREASE in the LEUCOCYTOSIS evoked by successive INTRAVENOUS INOCULATIONS of the same RABBIT with STAPHYLOCOCCUS AUREUS.



The diagram speaks for itself, but a word of comment is needed. It is seen that on the first occasion, the rabbit being then in an unprotected condition, a small dose of living cocci was overcome without the need for more than a trifling leucocytosis. On the second occasion a much larger quantum of cocci was also overcome, but the leucocytic response was more pronounced. On both these occasions the maximum leucocytosis occurred after the cocci had

disappeared from the circulating blood : it was not an immediate response to infection : some 48 hours elapsed before its acme was reached. But, in the case of the fourth infection, not only was the leucocytic response vastly in excess of anything previously seen, but it was also immediate. Within  $2\frac{1}{2}$  hours of inoculation the leucocytes numbered 24,000 per c. mm. and in  $9\frac{1}{2}$  hours, 51,000. The acme occurred soon after the cocci had vanished from the blood : then there was a fall, as abrupt as the rise, followed on the third day by a further rise which doubtless coincided with the maturation of the secondary foci of infection in the viscera. It is difficult to believe that the differences of leucocytic response seen in these successive infections depend merely on the different doses of cocci administered. To us it appears more probable that, in the process of immunisation the leucocytes, or rather the leucoblastic tissues (bone marrow), become educated to more prompt and effective reaction : and of this we have already obtained some evidence by microscopic examination of the marrow after death. The animal which was the subject of this experiment shewed, in contrast with the normal animal (inoculated at the same time and with the same dose, but which died in 24 hours with no leucocytosis), a marked leucoblastic reaction in the marrow of the femur.

4. It is a matter of regret that we have not, in this experiment, more complete data as to the behaviour of the opsonic index. In the two final infections, a rise is seen, in 11 days, from 1.5 to 2.0, but, in spite of the great leucocytosis, the high opsonic index at death, seemed to be without effect in averting the fatal issue.

CASE IV.—This experiment concerns a normal rabbit which served as a control in the last stage of the preceding experiment. It was inoculated on June 19th at the same time and with the same dose (0.5 mgm. living *Staphylococcus aureus*, 24 hour agar culture) as the immune rabbit. But whereas the immune rabbit survived for five days, this animal died in 25 hours.

TABLE XXV.—SHOWING the EFFECT of an INTRAVENOUS DOSE of *STAPHYLOCOCCUS AUREUS* upon a NORMAL RABBIT: DEATH in 25 HOURS.

Date.	Inoculation.	No. of cocci in 1/10 cc. blood.	No. of cocci in 1/100 cc. blood.	No. of cocci in 1/1000 cc. blood.	No. of leucocytes per c.mm. blood.	Weight in grammes.
June 19— 9.50 a.m. ...	0.5 mgm. living cocci.				2,600	2,430
12.0 ...		7	4	1 in 1/500 cc.	1,200	
2 p.m....		Several	2	0	6,400	
4.45 p.m. ...		0	0	0	3,600	
7 p.m....		1	0	0	2,800	
June 20— 11 a.m. ...		Uncountable.	Uncountable.	300 approx.		2,450

Seven hours after the intravenous injection, the cocci had vanished from the circulation, but next morning the animal was found moribund, and, just before death, cultures from the blood shewed the presence of no less than 300,000 cocci per cc. Counts of the leucocytes which were made shewed only a slight increase in numbers over the normal. This practical absence of leucocytosis in a rapidly fatal case coincides with what was seen in Case I., but it is noteworthy that even in absence of leucocytosis the cocci may temporarily disappear from the blood. This case calls for no further comment. Details are seen in the preceding table.

**CASES V, VI AND VII**—The three rabbits in this experiment may be considered together. The experiment concerns only the degree of leucocytosis to be observed in normal and immune rabbits similarly inoculated with the same dangerous dose of *Staphylococcus aureus*.

*Rabbit A.*—This was a normal adult rabbit, unprotected by any previous inoculation.

*Rabbit B.* (numbered "aureus 2" in our note book) had previously received the following immunising doses of *staphylococcus aureus* killed by heat:—

June 18th.	1.0 mgm.	killed cocci intravenously,
" 29th.	"	" " "
July 8th.	"	" " "
" 15th.	2.0 mgm.	" " "
Aug. 8th.	Half an	" " "
	agar culture.	

An interval of 53 days then elapsed, without any fresh inoculation up to the date of the present experiment.

*Rabbit C.* (numbered "aureus 8" in our case book) had previously received two immunising doses of the killed coccus intravenously:—

Aug. 14th.	1 mgm.	
" 23rd.	30 "	This enormous dose was administered in five successive doses, half an hour intervening between each. The animal was made very ill by this inoculation, but recovered. No further inoculation was performed between this and the present experiment—an interval of 38 days.

On September 30th the leucocytes of these three rabbits were first counted twice over, and an average was struck. As in all our leucocyte counts, 40 or 80 fields of the microscope were scrutinised in an ordinary Thoma-Zeiss hæmocytometer, the blood having been diluted 100 times with the red counter; 80 fields, having each a diameter of eight squares, correspond very closely to 1 c.mm. of the diluted blood. A mechanical stage was used to avoid the danger of counting the same field more than once. We are satisfied that the counts thus obtained are substantially accurate. It will be noted that at the commencement of the experiment rabbit C had a much higher leucocyte count than the other two animals;

it appeared also less lively than the others. Whether or not this was due to any residual mischief from the last immunisation we are unable to say; we could determine no other cause for the condition.\*

At 1.30 p.m. each rabbit received 0.5 mgm. of living staphylococcus aureus from a 24-hour agar culture administered intravenously. The leucocytes were then systematically counted from time to time with the results seen in the following table.

TABLE XXVI.—SHOWING the DEGREE of LEUCOCYTOSIS in a NORMAL and in TWO IMMUNE RABBITS after the ADMINISTRATION to each of 0.5 mgm. STAPHYLOCOCCUS CULTURE.

Date.		Leucocytes per c.mm. blood.		
		Rabbit A. (Normal.)	Rabbit B. (Immune.)	Rabbit C. (Immune.)
Sept. 30.	1 hour before inoculation ...	3,800	8,600	14,200
	At time of inoculation ...	4,800	6,400	17,800
	1 hour after inoculation ...	5,000	10,200	14,800
	2 hours after inoculation ...	6,000	—	21,000
	3 hours after inoculation ...	4,000	10,400	39,800
Oct. 1.	22 hours after inoculation ...	3,800	11,000	35,200
" 2	... ..	5,200	43,600	39,200
" 3	... ..	19,800	114,000	30,600
" 4	... ..	69,600	22,800	29,600
" 5	... ..	43,000	49,200	28,000
" 7	... ..	Death on Oct. 6.	69,600	34,600
" 9	... ..		Death on Oct. 8.	15,400
" 10	... ..			10,400 lively and well.

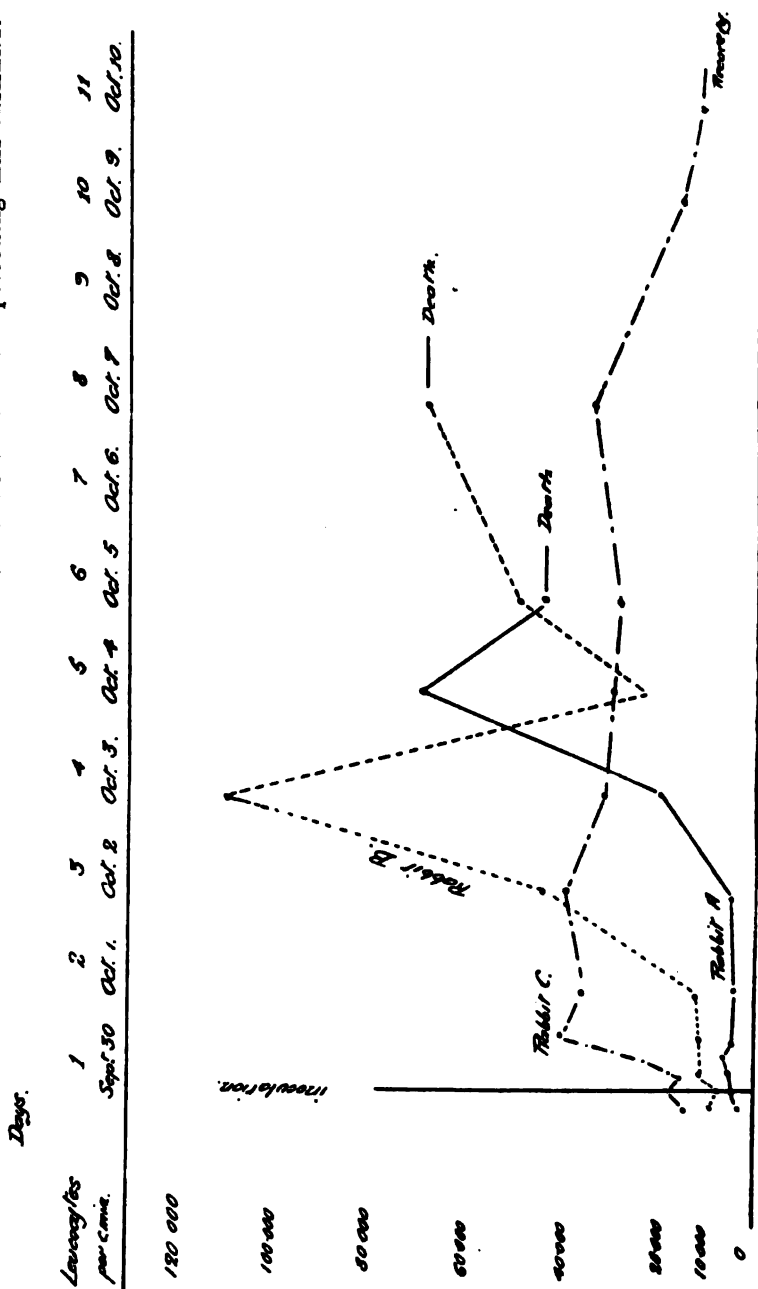
In rabbit A and rabbit B, succumbing in six and eight days respectively, the kidneys were the seat of suppurative nephritis; rabbit A had also suppurative myocarditis. Rabbit C recovered completely from its infection and remained well. The all-important question is why the one rabbit recovered while the other two died.

In order to give a clearer view of the course of the leucocytosis in the three animals we have plotted out the results to scale in the diagram subjoined.

It is seen in this diagram that the normal rabbit (A) showed a fair degree of resistance to the infection, life being prolonged to the end of the 6th day, which is in excess of the usual duration of

\* This animal was used for further immunisation experiments in the following year. It ultimately succumbed to suppurative meningitis following an intravenous injection of virulent Staphylococcus aureus, and it was then found that considerable mischief had followed its previous inoculations. Large caseous foci were found in connection with the sigmoid flexure of the colon and bronchial glands, and the left lung was completely atrophied.

DIAGRAM V.—EXHIBITING IN GRAPHIC FORM THE LEUCOCYTE CURVES IN THE PRECEDING EXPERIMENT.



life in an unprotected animal subjected to an intravenous dose of 0.5 mgm. of *Staphylococcus aureus* culture. Further, the animal showed a considerable leucocytosis, but not until the 5th and 6th days was there appreciable leucocytic response to the initial infection—for the first three days practically none at all was seen. The response coincided with the maturation of the secondary suppurative foci in the heart and kidneys and was, presumably, too late to avert a fatal issue.

The immunised animals, on the other hand, exhibited a much earlier leucocytic response to infection. In rabbit B the response is already high on the 3rd day, and by the 4th day is extreme; but it is transitory and not consistent; already on the 5th day there is a great fall in the leucocytosis, and the subsequent rise, which coincided with the development of the suppuration in the kidneys was inadequate to overcome the mischief which had arisen.

Rabbit C, which successfully resisted the infection, shows a marked difference to the other animals in two respects. The leucocytic response was far more prompt, being already high in three hours from infection; and although it was never so high as in the two other rabbits, the diagram shows that it was consistently maintained until the infection was overcome.

While it would be rash to draw too sweeping an inference from this one experiment, it is difficult to avoid the suspicion that rabbit C owed its life in part to its ability to put forth with promptitude and to maintain a consistent and fairly high degree of leucocytosis; this promptness and continuity of response would appear to have availed it more than the higher, but less ready and consistent, response seen in the other two animals. We have not, as yet, sufficient evidence to trace the reason for this efficient response, but our observations upon the bone marrow, at present in a very incomplete stage, induce us to suspect that in the education of this tissue may possibly be found a part of the secret of immunity. Another doubtless lies in the development of opsonin rendering the reinforced leucocytic response more available for defence, and yet another in the bactericidal action of the serum which we have described in Section II of this report. The relation of these and perhaps other factors in immunity against the pyogenic cocci is a matter worthy of more extended investigation.

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## APPENDIX B, No. 3.

## REPORT on the MICRO-ORGANISMS present in SEWER AIR and in the AIR of DRAINS; by F. W. ANDREWES, M.D.

## CONTENTS.

- I.—INTRODUCTION and HISTORICAL SUMMARY.  
 II.—SCOPE and METHODS of the INVESTIGATION.  
 III.—CHARACTERS of the STREPTOCOCCI of SEWER AIR and DRAIN AIR, and a COMPARISON of these with the STREPTOCOCCI of FRESH AIR and of SEWAGE respectively.  
 IV.—CHARACTERS of the BACILLI of the COLON GROUP from SEWER and DRAIN AIR, and from FRESH AIR and SEWAGE.  
 V.—EXPERIMENTS with BACILLUS PRODIGIOSUS BEARING on the CONVEYANCE of SEWAGE BACTERIA by SEWER AIR.  
 VI.—CONCLUSIONS.

## SECTION I.

## INTRODUCTION.

My instructions from the Board were "to undertake a bacteriological investigation, under varying conditions of sewer air and drain air, with reference to the biological properties of micro-organisms apt to become suspended in such air, and more particularly of such of them as may be found to belong to the streptococcus and *B. coli* groups."

As the result of earlier investigations the opinion has become current that, under ordinary conditions, sewage does not contaminate the air with which it is in contact with its own specific bacteria. The experiments upon which this view is based were conducted some ten or fifteen years ago, and the improvements which have in the mean time been effected in the technique appropriate to an inquiry of this kind have been such as to demand a re-investigation of the subject. The fundamental point which lies at the root of the whole sewer-air controversy is this: Does sewage specifically pollute the air of sewers and drains with its own bacteria, or does it not? To this matter I have devoted my whole attention.

## HISTORICAL SUMMARY.

With the introduction of water-carriage for the removal of excreta and household waste from dwellings arose the possibility of the escape of sewer air into houses from defective sanitary construction. Experience soon showed that where such escapes were observed they were liable to be associated with the occurrence of infectious disease; typhoid fever, scarlet fever,

diphtheria, septic pneumonia, severe tonsillitis, and puerperal fever are amongst the infections which have been found thus associated. The idea is firmly implanted in the public mind that the occurrence of one or other of these diseases in a household implies "something wrong with the drains," and although the evidence connecting the two is often inconclusive it may be said to have become an axiom in sanitary science that the escape of sewer air into houses introduces serious risk to health. It is still in dispute whether the sewer air actually conveys infectious disease, or merely predisposes to infection from other sources.

The progress of bacteriology has defined the nature of the infecting agent in most of the diseases which have been attributed to sewer air. The conclusive proof that such diseases are actually conveyed by sewer air must rest on the demonstration in such air of the known agents of infection. In no single case has such proof ever been forthcoming; the evidence has always been merely circumstantial. It has been argued that sewer men, who breathe undiluted sewer air during their working hours, do not appear more subject than others to zymotic disease; the argument has been met by the assumption that they become acclimatised or immunised by constant exposure. Such is a short statement of what may be termed the clinical aspect of the sewer-air problem.

The observations which have been made upon sewer air from the experimental side have been both chemical and bacteriological. Chemical analysis has revealed some excess of carbon dioxide in sewer air; where the sewer or drain is ill-ventilated the excess may be considerable. Traces of sulphuretted hydrogen have also been found. While there can be no doubt that cases of acute mephitic poisoning in foul sewers may be explained by accumulations of noxious gases, it is not too much to say that chemistry has revealed nothing in ordinary sewer air which can account for its deleterious effect, when, largely diluted, it escapes into dwelling houses.

The earliest investigations of the bacteria of sewer air are those of Levy and Miquel, in Paris, who commenced systematic observations in 1891. They established the fact that sewer air contains only about half as many micro-organisms as fresh air examined at the same time. In Berlin, Petri and also Uffelmann confirmed this observation; Uffelmann further determined the nature of many of the bacteria he found in sewer air and drain air; amongst those which he recognised were *Proteus vulgaris* and *Staphylococcus aureus*.

In this country the first investigators were Carnelley and Haldane, who carried out observations in the sewers of Dundee, Westminster, and Bristol. They did not concern themselves with the species of bacteria present in the sewer air, but they confirmed the conclusion that the number of bacteria was small in comparison with those of fresh air—averaging but 8.9 germs per litre—and they established the important fact that the larger the amount of carbonic acid present (*i.e.*, the worse the ventilation) the smaller is the number of bacteria in the air of a sewer. They considered that the bacteria in sewer air were derived from the fresh air outside.

In 1892 and 1893 appeared the reports to the London County Council on the Bacteria of Sewer Air, by Mr. J. Parry Laws. His general conclusions were as follows:—(1) the number of micro-organisms in sewer air (in the King's Scholars' Pond Sewer, Westminster) is smaller than in the fresh air examined at the same time; (2) the species of micro-organisms in sewer air are identical with those of fresh air, and not with those of sewage; (3) moderate splashing does not influence the bacterial content of sewer air, even within four feet of the disturbance; (4) stagnant and putrescent sewage does not influence the organisms in sewer air. In a further report to the London County Council, in 1894, on the Bacteriology of Sewage, I was associated with Mr. Laws; we were unable, by the methods then available, to find any evidence that sewage gives up any of its bacteria to the air in contact with it.

Up to this time, therefore, there was practical unanimity amongst those who had studied the bacteriology of sewer air as to certain facts. Sewer air was shown to contain actually fewer bacteria than fresh air, and those bacteria found were shown to correspond in the main with the species demonstrable in fresh air. It is upon these data that the current opinion has been founded, that, apart perhaps from violent splashing, sewage does not give up its contained bacteria to sewer air. As a corollary to this view, it has become necessary to explain the observed ill-effects of sewer air when this escapes into a dwelling, as dependent upon some subtle chemical constituent which undermines the health and predisposes to extraneous infection.

### *Criticism of the foregoing Facts.*

While the facts just mentioned are established beyond dispute, it may be doubted whether they express the whole truth. The history of our knowledge with regard to the bacteriology of water may serve as a warning in this connection. So long as cultures were made from only one cubic centimetre the organisms to which so much importance is now attached practically escaped detection, while the isolation of pathogenic microbes was an almost hopeless task. But with the introduction of selective media it became possible to employ much larger volumes of water for cultures, and our knowledge was greatly advanced. Similar methods have already effected a marked improvement in our knowledge of the microbes of air: thus Gordon has shown how, by using phenol-broth plates and incubating the fluid which has been exposed under anaërobic conditions, streptococci can readily be obtained from air, though under the old methods they could hardly ever be detected. It is probable that the use of media appropriate to the organism sought for, but inappropriate to the common bacteria of fresh air, will do for the examination of air what it has already done for that of water. Chemical and biological tests have been introduced which enable us to recognise given forms of bacteria with an ease and certainty undreamed of ten years ago. Tasks which in 1894 were beyond the power of bacteriologists may now be undertaken with much greater prospects of success.

*Explanation of the Poverty of Sewer Air in Bacteria.*

It will be useful at this point to consider the reasons for the small number of micro-organisms in sewer air. Dr. W. H. Hurtley has put these very plainly in his joint report with me to the Hampstead Borough Council in 1905. Sewer air is always saturated, or nearly so, with water vapour; even if a small diminution of temperature occurs, condensation of this vapour takes place, and most readily round any particulate matter, such as bacteria, which may be suspended in the air; the germs act as nuclei for the deposit of dew and are hence removed by precipitation, falling into the sewage beneath or becoming adherent to the moist walls of the sewer. Bacteria introduced by ventilation from the fresh air are thus soon reduced in number within a sewer, and unless replenishment readily occurs from sources within the sewer itself the number must remain small. Under the conditions of quietly flowing sewage in a main sewer—and it is under these conditions that observations have mostly been conducted in the past—such replenishment does not take place to any appreciable extent. Particulate matter is not readily disengaged from moist surfaces. The walls of a sewer are practically always moist, and it has been shown that it takes a strong air current to dislodge particles from the surface of a fluid. Practically, then, we have a complete physical explanation for the observed facts that sewer air contains but few microbes, and these mostly derived from the fresh air, and that the fouler and worse ventilated the sewer, the fewer the microbes.

Nevertheless conditions have long been remarked under which particles of sewage might be disengaged from its surface. These are: (1) the *bursting of bubbles*, in the escape of gas from putrescent sewage, and (2) *splashing*, where a lateral outfall enters a main sewer. Laws, who used only 10 litres of air for each observation, was unable to detect the influence of these conditions. I shall later give reasons for believing that the influence of splashing is by no means unimportant in this connection, and that in relation to house drains, discharging from a height into an inspection chamber, it may play a considerable part in disseminating sewage bacteria. Quite recently Major Horrocks has called attention to this, and has suggested a third way by which sewage bacteria may get into the air of sewers and drains, namely, by the alternate moistening and drying of the walls where the flow of sewage is intermittent—a condition especially apt to occur in small drains. It is probable that bacteria are easily detached from a dry surface.

*Previous Investigations of Sewer Air by the Aid of Selective Culture Media.*

In the year 1905 I was requested by the Hampstead Borough Council to investigate sewer emanations from ventilating-grids in the roads, as regards their possible harmfulness, and I had the advantage of collaboration with Dr. W. H. Hurtley on the chemical side of the question. Instead of examining such small quantities of sewer air as 10 litres, I exposed plates of culture media in the sewers for an hour. No quantitative result was thus obtainable,

but this was of little moment, for I wished to determine the correctness of the view that sewer air never gave up its bacteria to the air with which it was in contact under the conditions prevailing in modern sewers. As the result of an experiment carried out for the purpose, I estimated that the exposure of a plate culture,  $4\frac{1}{2}$  inches in diameter, for one hour, yielded colonies corresponding to the bacterial content of several hundred litres of sewer air. Overgrowth of the plates by moulds and ordinary air bacteria was obviated by using selective media capable of preventing their growth, while favouring that of the organisms for which I was in search (phenol-broth, and Conradi and Drigalski's medium). I had determined that the sewage in the main sewers of Hampstead contained some 5 million organisms per cc. Of these the most abundant were streptococci, which numbered over a million per cc., while members of the *B. coli* group were present to about a quarter of a million per cc. In all cases the experiments were controlled by similar observations carried out at the same time in the fresh air.

The results which I obtained were shortly as follows : In the sewer air I found *B. coli communis* once, and on another occasion another member of the *B. coli* group, present also in the sewage. I also once found *B. coli communis* in the fresh air. Streptococci were obtained readily from the sewer air, and a comparison of their biological characters with those obtained from sewage and from fresh air showed that they corresponded much more closely with the former than with the latter. The observations were limited in number, but they sufficed to make out a *primâ-facie* case for reconsideration of the opinion that sewage organisms are not to be found in sewer air.

I may add here that the brilliant observations which Major Horrocks has carried out at Gibraltar, first published in 1907, and of which I was unaware till the experiments recorded in this report were nearly completed, have most fully established the readiness with which, under certain conditions, micro-organisms may escape from sewage into sewer air.

## SECTION II.

### THE SCOPE AND METHODS OF THE PRESENT INVESTIGATION.

The primary aim of the investigation was to prove or disprove the truth of the view hitherto current, that sewage does not, under ordinary circumstances, give up its bacteria to the air with which it is in contact. My Hampstead experiments had suggested that this view was erroneous, but for final proof of the matter more extended observations were necessary. According to my instructions from the Board I made special search for members of the streptococcus and *B. coli* groups. Not only are these organisms the most abundant and characteristic organisms of sewage, but they have been, during the last few years, the subjects of special study, so that I was in a position to apply to such as I might find a number of biological tests which would enable them to be differentiated with a greater precision than has hitherto been possible.

The importance of such minute study is evident from the following consideration. It is required to prove that certain organisms in sewer air are of human faecal origin and not derived from fresh air. But the fresh air of towns, especially in dry and windy weather, is extensively polluted by dried horse-dung. It has, therefore, to be shown that sewer air contains microbes which are not found in fresh air, or that it contains them in much larger number than does the fresh air, and that such microbes are characteristic of sewage rather than of horse-dung.

With regard to the *B. coli* group, we have no sufficient body of evidence as to the relative frequency of the different forms in human and in equine faeces. I have therefore had to rely upon the relative biological characters of the members of this group as found respectively in sewage, in sewer air, and in fresh air. As a selective culture medium I have largely used that devised by Conradi and Drigalski, which has the double advantage that it inhibits the growth of most of the common air organisms, while permitting free growth of members of the *B. coli* group and of most streptococci, and that the various colon bacilli grow in characteristic fashion, so that the individual species can, in part, be recognized by the naked eye appearance of their colonies. I have also derived great assistance from the work of Dr. MacConkey, who has not only devised selective media of almost equal value to that of Conradi and Drigalski, but has also introduced a series of biological tests by which the differentiation of members of the *B. coli* group has been simplified and rendered more precise.

As regards the group of Streptococci I have employed the tests put forward by Dr. M. H. Gordon in his report to the Board (1903-4). In 1906 I published in the *Lancet*, in conjunction with Dr. T. J. Horder, an analysis of more than 1,200 strains of streptococci in which Gordon's tests had been employed: in this analysis an attempt was for the first time made to distinguish provisional groups of streptococci according to their biological characters and to trace such groups to their natural habitat. I have found the conclusions to which we were led of considerable assistance in the present investigation.

Doubt has been felt by some as to the value of fermentation tests in distinguishing between allied species of bacteria. Mr. F. W. Twort has proved (*Proc. Royal Soc.* 1907) that if certain members of the *B. coli* group are grown for many generations in a medium containing a test substance which they are originally unable to ferment, they may in time acquire the power of fermenting the substance. Thus he succeeded in inducing the typhoid bacillus to produce an acid reaction both with lactose and dulcitate. While it must be admitted that the term "species" must be used with caution in speaking of bacteria, it can hardly be said that Twort's interesting facts seriously diminish the value of such tests as Gordon's or MacConkey's. For we judge of differential characters, not by what an organism can be forced to do under abnormal, but by what it habitually does under ordinary conditions. I remain content to use these fermentation tests as the best guide at present available for discriminating between the various natural types met with amongst the *B. coli* group and the Streptococci.

Most of the observations which have been carried out on sewer air have been devoted to the air of main sewers. For this reason, amongst others, I have chiefly concerned myself with drain-air, about which much less is known. As I was working in the laboratories of St. Bartholomew's Hospital, I naturally selected the drains of that institution, not only for convenience but because they were admirably suited to my purpose. The drainage of the Hospital was carried out anew some eighteen years ago and is a good example of modern sanitary work. The drains are frequently inspected, and are clean and in good condition. The largest outfall from the Hospital is into the sewer in Little Britain. The drainage from a considerable area, including about half the wards and the nurses' home, is here collected into an 18-inch pipe, which, narrowing somewhat at the actual outfall, discharges by an ordinary interceptor into the sewer outside. Some 60 feet above the outfall two lateral drains open into the main channel, with a drop of a few inches, giving rise to a slight degree of splashing. The flow of sewage is continuous and fairly rapid, the gradient being good. There is some gurgling, but no visible splashing, as the sewage passes through the interceptor. Just before the outfall there is access to the drain by a manhole and arched brick passage floored with cement: the ventilation here, as elsewhere in the hospital drains, is good and no unpleasant smell is noticeable. My experiments have chiefly been carried out in the arched passage leading from the manhole to the drain as above described. I was able to expose culture plates at any desired distance from the flowing sewage, from 2 up to 12 feet or more. When I refer to the "Little Britain outfall" I mean this spot.

It will be convenient to record the results I have obtained, not in their chronological order, but according to the special micro-organisms under consideration. The evidence will thus gain in clearness.

### SECTION III.

#### EVIDENCE AFFORDED BY THE BIOLOGICAL CHARACTERS OF THE STREPTOCOCCI OF DRAIN AND SEWER AIR AS TO THEIR ORIGIN.

The observations on this point have involved a study of the characters of the Streptococci in fresh air and in sewage, as well as of those obtained from sewer and drain air. In order to render the evidence as complete as possible I shall incorporate with the results obtained in these observations some which I have arrived at in previous experiments and some which I have derived from other sources, in each case stating the authority which I quote. In examining the biological characters of the streptococci I have, with one exception, used the tests devised by Dr. Gordon; the exception is the coniferin test. This is, in my experience, the least valuable of Gordon's tests, and it was not employed by Dr. Houston in his work on the Streptococci of Human Fæces (Report of the Medical Officer, 1903-4); further, the stock of coniferin in the European market has been so depleted that the substance has been of late unprocureable. I have found it fairly easy to obtain streptococci from air by exposing plates of Conradi and

Drigalski's medium, though many of the minute colonies which develop on such plates prove on examination to be of other nature. I have also used the more sensitive method of exposing broth-plates containing 0·05 per cent. phenol, and incubating the fluid anaërobically; the growth obtained was plated out on agar, and individual colonies were picked out for sub-culture.

Before recording my results, I may refer to the conclusions reached in the analysis, to which I have already referred, by Dr. Horder and myself, of the reactions of all the available streptococci which have been submitted to Gordon's tests, over 1,200 in number, and to the tentative grouping and nomenclature which we have suggested for the streptococcal types commonly met with. We have carefully guarded ourselves against putting forward any types as fixed and rigid species: on the contrary we found such infinite variability in chemical reactions as to lead rather to the belief that specific limits were ill-defined and variable amongst the streptococci. We therefore assigned provisional names to those chemical types which recurred with such frequency as to make it convenient to have a name for them. The terminology is as follows:—

- (1) *Streptococcus equinus*.—A saprophyte indigenous in horse dung, and occurring also in the human intestine. Characterised by inability to ferment lactose, and even to give an acid reaction in milk: the usual positive tests in Gordon's series were with saccharose and the two glucosides (salicin and coniferin).
- (2) *Streptococcus mitis*.—A short chained saprophyte common in human saliva and fæces. It forms acid in milk but gives no clot: lactose is fermented as well as saccharose and the glucosides.
- (3) *Streptococcus pyogenes*.—The long chained pathogenic form of chief importance in human disease-processes. Milk is acidified but not clotted: the characteristic positive reactions are saccharose, lactose, and salicin mannite may be added.
- (4) *Streptococcus salivarius*.—A short chained form, typically found in the mouth, where it is the most abundant organism; it is also common in the intestine. Occasionally pathogenic for man, causing terminal infections, malignant endocarditis, &c. The characteristic reactions are clotting of milk, reduction of neutral red, and fermentation of saccharose, lactose, and often raffinose; salicin may be added.
- (5) *Streptococcus anginosus*.—Identical in its reactions with the preceding, but long chained and more definitely pathogenic.
- (6) *Streptococcus faecalis*.—A short chained saprophyte, eminently characteristic of human fæces. It reacts positively to all of Gordon's nine tests, except raffinose and inulin.
- (7) *The Pneumococcus*.

In addition to these main types we found numerous intermediate forms which we classed as varieties of one or other main type. I shall employ this terminology in what follows.

*The Streptococci of Fresh Air (in London).*

Plates of Conradi and Drigalski's medium were exposed on various occasions, usually for an hour, about the precincts of St. Bartholomew's Hospital and on the vacant site of Christ's Hospital: sub-cultures were then made (after incubation of the plates for one or two days at 37° C.) from such minute colonies as seemed likely to be streptococci. Only a small proportion of such minute colonies proved to be streptococci: *e.g.*, in one experiment only four cultures of streptococci were obtained from 30 colonies sub-cultured. The reactions of eight strains of streptococci thus obtained are seen in the following table:—

TABLE I.—REACTIONS of STREPTOCOCCI obtained from FRESH AIR in the CITY of LONDON.

Streptococcus.	Litmus milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Growth on gelatin at 20° C.	Length of chain in broth.	Type.
	Acid.	Clot.										
1	•	•	•	+	•	•	•	+	•	—	Medius	Equinus.
2	•	•	•	+	•	•	•	+	•	—	Medius	Equinus.
3	•	•	•	•	•	•	+	+	•	—	Medius	Equinus var.
4	•	•	•	•	•	+	•	+	•	+	Longus	?
5	•	•	•	+	•	•	•	+	•	—	Medius	Equinus.
6	+	+	•	+	+	•	•	+	•	+	Brevis	Salivarius var
7	•	•	•	+	•	•	•	+	•	—	Medius	Equinus.
8	+	+	•	+	•	+	•	•	+	+	Brevis	Fæcalis var.

Having obtained these results I desisted from further experiments upon fresh air streptococci, because it was evident that the streptococci in the air of the City were substantially identical with those found elsewhere in London. At Hampstead I had tested 16 colonies of streptococci in fresh air, and had found 10 of them to correspond to the equinus type and three to the fæcalis type, while three were of doubtful nature. Gordon had reported on a much longer series of colonies tested by him in his report on the ventilation of the House of Commons, some 40 in number, and a critical analysis of these yielded similar results. It may, therefore, be regarded as established that of the streptococci to be obtained from fresh air in London a great majority (65 per cent. of the 64 recorded strains) belong to the equinus type and are presumably derived from horse dung, in which I have found this type present up to 10 million per gramme. In smaller numbers, members of the mitis type (3 per cent.), of the salivarius type (6 per cent.), and of the fæcalis type (20 per cent.) also occur, while there are a few aberrant forms which do not fall in with any definite type.

*The Streptococci of Sewage.*

My observations on the fresh sewage leaving St. Bartholomew's Hospital show that streptococci are the most abundant organisms

therein. I have on several occasions carried out decimal dilutions of the sewage up to one part in 10 million, and have found that streptococci can be obtained from the higher dilutions which no longer yield any growth of *Bacillus coli* or its allies. In this sewage I have found that streptococci number, on different occasions, from 100,000 to 10,000,000 per cc. This accords well with the results which I obtained from a main sewer in Hampstead; in this, which fairly represents the mixed sewage of a residential district, streptococci numbered over 1,000,000 per cc. of sewage, considerably outnumbering all other organisms.

In the present series of experiments I have submitted ten colonies taken at random from plates prepared from high dilutions of St. Bartholomew's sewage to Gordon's series of tests. With one or two exceptions, which failed to clot milk and are hence to be referred to varieties of the mitis type, these strains are all examples of streptococcus salivarius and of its salicin-fermenting variety which must hence be regarded as the typical form of streptococcus in this local sewage. Five out of nine forms isolated from mixed Hampstead sewage fall under the same category. I give the reactions of these nineteen strains of sewage streptococci in the following table:—

TABLE II.—REACTIONS of STREPTOCOCCI obtained from SEWAGE (CITY and HAMPSTEAD).

Number of times met with.	Litmus milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Growth on gelatine at 20° C.	Length of chain in broth.	Type.
	Acid.	Clot.										
1 colony ..	•	•	+	+	•	•	•	•	•	—	Longus	Equinus var.
3 colonies ..	+	•	+	+	+	+	•	•	•	+	Brevis	Mitis var.
1 colony ..	+	•	+	•	+	•	+	•	•		Brevis	Mitis var.
2 colonies ..	+	+	+	+	+	+	•	•	•	±	Brevis	Salivarius.
1 colony ..	+	+	+	+	+	•	•	•	•	+	Brevis	Salivarius.
1 colony ..	+	+	+	+	+	+	•	+	•	—	Brevis	Salivarius var.
1 colony ..	+	+	•	+	+	+	•	•	•	+	Brevis	Salivarius var.
1 colony ..	+	+	+	+	+	•	•	+	•	+	Brevis	Salivarius var.
4 colonies ..	+	+	±	+	+	•	•	+	•	+	Brevis	Salivarius var.
1 colony ..	+	+	•	+	+	+	•	+	•	+	Brevis	Salivarius var.
1 colony ..	+	+	+	+	+	•	•	•	+	+	Longus	Fæcalis var.
1 colony ..	+	+	+	+	+	+	•	•	+	+	Longus	Fæcalis var.
1 colony ..	+	•	+	+	+	+	•	+	+	+	Longus	Fæcalis var.

It will be seen from this table that the streptococci most abundant in sewage are of very different type from those met with in the fresh air. The equinus type forms here but 5·2 per cent. of the total, whereas the salivarius type constitutes some 58 per cent.

*The Streptococci of Sewer and Drain Air.*

Out of a large number of minute colonies sub-cultivated from plates of Conradi and Drigalski's medium, exposed at different times in the drains at St. Bartholomew's Hospital, some for an hour, some for half-an-hour, I obtained only six strains of streptococci. These, without exception, belonged to the salivarius type. The sewer air streptococci which I had previously obtained at Hampstead, 13 strains in number, had belonged, eight to the salivarius and five to the faecalis type. No one of the 19 strains from sewer and drain air could be referred to the equinus type which I have shown to be the preponderating form in the fresh air of London. In the following table I record the reactions of the 19 strains of streptococci I have obtained from sewer and drain air.

TABLE III.—REACTIONS of STREPTOCOCCI obtained from SEWER AIR and DRAIN AIR (CITY and HAMPSTEAD).

Number of times met with.	Litmus milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Growth on gelatine at 20° C.	Length of chain in broth.	Type.
	Acid.	Clot.										
6 colonies ..	+	+	+	+	+	•	•	+	•	+	Brevis	Salivarius var.
3 colonies ..	+	+	+	•	+	•	•	+	•	+	Brevis	Salivarius var.
4 colonies ..	+	+	+	+	+	+	•	•	•	+	Brevis	Salivarius var.
1 colony ..	+	+	+	+	+	+	•	+	•		Brevis	Salivarius var.
2 colonies ..	+	+	+	+	+	•	•	+	+	+	Brevis	Faecalis type.
1 colony ..	+	+	•	+	+	•	•	+	+	+	Brevis	Faecalis var.
2 colonies ..	+	+	+	+	+	+	•	+	+	+	Brevis	Faecalis var.

The streptococci from sewer air and drain air are seen to bear a much closer family resemblance to those of sewage than to those of fresh air, the chemical reactions being in some cases identical. In the next table I summarise the percentage distribution of the streptococci from the three sources amongst the various types.

TABLE IV.—PERCENTAGE DISTRIBUTION of the STREPTOCOCCI of FRESH AIR, SEWER and DRAIN AIR, and SEWAGE, amongst the FOUR different TYPES concerned.

Type of Streptococcus.				Fresh air.	Sewer air.	Sewage.
				Per cent.	Per cent.	Per cent.
Equinus	...	...	...	65·6	0·	5·3
Mitis	...	...	...	4·7	0·	21·1
Salivarius	...	...	...	9·4	73·6	57·9
Faecalis	...	...	...	20·3	26·4	15·7

In calculating the percentage distribution of the fresh air streptococci I have used the material published by Gordon, as well as my own results. The other two columns are based on my own results in the City and at Hampstead. While I freely admit that the "types" of streptococci on which I base the figures are arbitrary and do not necessarily represent distinct species, the differences which they imply are, I believe, real.

*Conclusions as to the Evidence afforded by the Streptococci of Sewer and Drain Air.*

- (1) I have shown that the most abundant and characteristic streptococcus of the fresh air of London is the type which I have called *Streptococcus equinus*; my own results are at one with those of Gordon as to this. I have failed to recognise this type in the air of drains and sewers.
- (2) I have shown that the most abundant and characteristic streptococcus of sewage, in the City and in Hampstead, belongs to the type which I have distinguished as *Streptococcus salivarius*.
- (3) I have shown that 19 streptococci from sewer and drain air correspond mainly with the *salivarius* type, but in part to the characteristic streptococcus found by Houston in human faeces.

The conclusion appears irresistible that the streptococci to be found in sewer and drain air are, in the main, not derived from the fresh air by processes of ventilation, but are derived in some way from the sewage itself.

#### SECTION IV.

**EVIDENCE AFFORDED BY THE BIOLOGICAL CHARACTERS OF THE MEMBERS OF THE BACILLUS COLI GROUP TO BE FOUND IN SEWER AND DRAIN AIR, AS TO THEIR ORIGIN.**

It is a matter for some surprise that, whereas *Bacillus coli* and its allies are present in horse dung, according to my observations, to the number of some 10,000 per gramme, they should rarely be demonstrable in the fresh air of towns, in which dried horse dung is so abundant. Most of those who have sought for this bacillus in air have failed to find it, even though considerable volumes have been examined. In experiments which I carried out in 1902 for the London County Council on the air of the Central London Railway, I was unable to detect any member of the *B. coli* group in 10 litre samples of air taken on 12 occasions from fresh air and on 12 occasions from the air of the railway. At Hampstead, in 1905, I once obtained the classical *B. coli communis*, on a dry and windy day from a phenol-broth plate exposed for an hour in the fresh air. In the present series of experiments, carried out in and near St. Bartholomew's Hospital, I have never found the classical *B. coli communis* in fresh air, though I have occasionally found other members of the group. It is a very striking fact that it is

possible to expose large plates of Conradi and Drigalski's medium for an hour on a dry and windy day in the centre of London without obtaining any colonies of *B. coli communis*, even though the plates become littered with particles of straw and débris conveyed by the wind.

### *Colon Bacilli in Fresh Air.*

In the course of the present experiments I have, on seven different occasions exposed large plates, 6 inches in diameter, containing appropriate differential culture media to the fresh air in or near the precincts of St. Bartholomew's Hospital. The media have been (a) Conradi and Drigalski's medium; (b) MacConkey's bile-salt, neutral red lactose agar; (c) lactose litmus broth. The times of exposure have usually been one hour, but on two occasions only half-an-hour. The exposures have been made under very varied atmospheric conditions, in calm weather and in high wind, in bright sunshine and in a black London fog, but not during actual rain. The plates have often been covered by smuts, fragments of straw and other débris. A list of the exposures is as follows:—

- (1) *January 19th.*—Two plates of Conradi and Drigalski's medium, exposed for one hour on the waste site of Christ's Hospital.
- (2) *January 21st.*—One plate of Conradi and Drigalski's medium, exposed for one hour on a window-ledge near the Little Britain entrance to the hospital.
- (3) *February 20th.*—Two plates of Conradi and Drigalski's medium, and one plate of bile-salt neutral red lactose agar, exposed for one hour in an open space outside the nurses' home.
- (4) *February 28th.*—Two plates of bile-salt neutral red lactose agar, exposed for an hour during a dense fog on the roof of the new out-patient block.
- (5) *June 14th.*—One plate of Conradi and Drigalski's medium, and one plate of bile-salt neutral red lactose agar, exposed for 30 minutes on a window-ledge near the Little Britain entrance to the hospital.
- (6) *September 25th.*—Three plates of lactose litmus broth, exposed in the same situation as No. 5, for 30, 45 and 60 minutes respectively. After incubation these broths were plated out on Conradi and Drigalski's medium.
- (7) *November 22nd.*—Two plates of Conradi and Drigalski's medium, exposed for one hour in the same situation as the last two experiments. (In this case small 4-inch plates were used.)

*Result.*—In no plate did any colony of *B. coli communis* appear. On one occasion I obtained a member of the *B. coli* group, which fermented lactose, clotted milk, and formed gas in gelatin shake culture, but it differed from *B. coli communis* in being unable to ferment dulcitol, to form indol or to reduce neutral red. On two

other occasions I obtained a bacillus identical in all its reactions with *B. lactis aërogenes*. *These three colonies represent the total result, as regards members of the B. coli group, of all the above exposures.* Many colonies of other sorts grew on plates of Conradi and Drigalski's medium, but they proved to be bacilli unable to ferment lactose, and in other respects no close relatives of *B. coli*.

It follows from these observations that *B. coli communis* and its near allies are of very sparse distribution in the air of the City of London. If, then, as I shall presently show, bacteria of this group are readily demonstrable in the air of drains, it is evident that their source is not the fresh air outside.

*Biological Reactions of the Colon bacilli and their allies found in fresh Sewage.*

When I investigated the bacteria of sewage at Hampstead in 1905, I employed as tests for *B. coli communis* the following:—

Gas formation in gelatin shake cultures,  
Acid formation and clotting in litmus milk,  
Reduction of neutral red,  
Gas formation in glucose bile-salt litmus broth,  
Gas formation in lactose bile-salt litmus broth,  
Indol formation in peptone broth.

Many of the colonies obtained from high dilutions of the sewage reacted positively to all these tests, and were classed, probably in most cases correctly, as *B. coli communis*.

Dr. MacConkey, in a paper on the Bacteriology of Milk ("Journal of Hygiene," Vol. VI., p. 385, 1906), has suggested tests by which the members of the *B. coli* group can be more sharply differentiated. He employs bile-salt litmus broth containing lactose, saccharose, dulcitate, adonite and inulin respectively, together with Voges and Proskauer's reaction (production of a reddish colour on the addition of caustic potash or soda to a glucose peptone culture of the organism to be tested). I have availed myself of these tests in examining sewage and drain air in the present series of experiments, and I have no hesitation in confirming MacConkey's claim as to the sharper differentiation to be obtained by their use. Without them one may easily confound together *B. coli communis*, *B. lactis aërogenes* and *B. cloacæ*; with them, these organisms are promptly and readily distinguished.

In order to determine the members of the *B. coli* group most abundantly present in the fresh sewage at the Little Britain out-fall of St. Bartholomew's Hospital, where I chiefly examined the drain air, I carried out the following observation:—

A sample of the sewage was subjected to decimal dilution, and from the dilutions plates were prepared on Conradi and Drigalski's medium and on bile salt neutral red lactose agar. From the colonies which arose a number were selected for subculture as diverse as possible in their naked eye characters. The subcultures were then submitted to the various tests. All the organisms tested were present to the number of over 3,000 per cc. of sewage. The reactions obtained are shown in the following table, together

with the species or group to which the organism is to be referred :—

TABLE V.—REACTIONS of BACILLI of the COLON GROUP from FRESH SEWAGE.

Number.	Bile-salt litmus brotha.					Voges and Proskauer's reaction.	Motility.	Liquefaction of gelatin.	Gas in gelatin shake culture.	Litmus milk.		Neutral red.	Indol.	Species.
	Lactose.	Saccharose.	Dulcitate.	Adonite.	Inulin.					Acid.	Clot.			
1	•	•	•	•	•	•	•	•	•	•	•	trace	•	Species unidentified.
2	+	+	+	•	•	•	•	•	+	+	+	trace	•	Atypical coli.
3	+	+	+	•	•	•	+	•	+	+	+	+	•	Atypical coli.
4	+	+	+	•	•	•	+	•	+	+	+	•	•	Atypical coli.
5	+	•	•	•	•	•	•	•	+	+	+	+	+	Atypical coli.
6	+	+	•	+	•	•	•	•	+	+	+	+	•	<i>B. lactis aërogenes</i> .
7	+	+	•	+	•	•	•	•	+	+	+	+	+	Atypical <i>B. lactis aërogenes</i> .
8	+	+	•	trace	•	•	•	•	+	+	+	trace	•	Atypical <i>B. lactis aërogenes</i> .
9	+	+	+	+	•	+	•	•	+	+	+	+	+	Friedländer group.
10	+	+	•	•	•	+	+	+	+	+	+	+	•	<i>B. cloacæ</i> .
11	+	+	•	•	•	+	•	?	+	+	+	+	•	Atypical <i>B. cloacæ</i> .

In this experiment it will be seen that no typical *B. coli communis* was found amongst the colonies subcultivated, though, had I not used MacConkey's tests, Nos. 5, 7 and 9 would have passed as such. No. 1 is not a true member of the coli group. The others are sufficiently described in the last column of the table, in which I have employed MacConkey's data for identification. I could doubtless have isolated the classical *B. coli communis* had I repeated the experiment, but this seemed unnecessary as its occurrence in sewage is undisputed. I merely desired to ascertain the commoner types of bacilli of the colon group in the sewage at the site of my experiments on the air of the drain.

*Numbers and Biological Characters of Bacilli of the Colon Group obtained from Drain Air at St. Bartholomew's Hospital.*

I have on several different occasions exposed plates of suitable culture media to the drain air at the Little Britain outfall, at St. Bartholomew's Hospital, but one experiment was carried out on a more extensive scale than the rest, and yielded material so rich that three or four weeks were occupied in fully investigating it. I will describe the results of this experiment in detail.

On January 21st, 1907, I exposed for an hour, in the situation previously described, within a few feet of the flowing sewage, nine

six-inch plates. One plate contained Conradi and Drigalski's medium; the others contained a bile-salt neutral-red agar medium impregnated with 1 per cent. of eight different test substances respectively, viz., glucose, saccharose, lactose, raffinose, salicin, glycerin, mannite, and isodulcit. At the same time a control Conradi and Drigalski plate was exposed for the same time in the fresh air adjacent, and from this no member of the *B. coli* group could be isolated.

The *Conradi and Drigalski* plate exposed in the drain showed, on the other hand, no less than 30 red colonies superficially resembling *B. coli*, as well as many blue or neutral colonies. Fifteen of the red colonies were selected for subculture.

The various bile salt neutral-red plates yielded the following results (it is to be noted that a red colony on such plates offers presumptive evidence that the organism can ferment the test substance present):—

The *glucose plate* yielded 76 red colonies and 41 colourless ones. (10 of these were subcultivated.)

The *saccharose plate* yielded 32 large red colonies, 29 large colourless ones, and 63 small colonies. (None of these small colonies were streptococci, so far as they were examined; indeed no streptococcus was obtained from any bile-salt medium in the course of these experiments; it would appear that the sodium taurocholate inhibits their growth.)

The *lactose plate* yielded 36 red colonies, 27 large colourless ones and 43 small colonies.

The *isodulcit plate* yielded 34 red colonies, 16 large colourless ones and a few small ones.

The colonies were not counted on the remaining four plates. On the *glycerin and mannite plates* they were fully as numerous as on the preceding four, many being red colonies. The *raffinose* and *salicin* plates had fewer colonies, but some of these were red. Altogether 44 colonies of coliform bacilli were cultivated from this set of plates and subjected to further tests.

Each subculture was first submitted to fermentation tests with saccharose, dulcitate, adonite and inulin, and to Voges and Proskauer's test: the motility of the bacilli was also determined in the hanging drop, but the question of what constitutes true motility is not easy to answer. I have recorded as motile only those instances in which the movement was active and beyond doubt. Liquefaction of gelatin was noted in some cases after 3 weeks or a month; no rapidly liquefying types have been included in the series; two cultures were rejected on this account. It is unnecessary to record in detail the results of these preliminary tests. They served to reduce the number of possible varieties present to 20. These 20 were now subjected to further tests, viz.:—

- Gas formation in ordinary gelatin shake-cultures.
- Litmus milk, for acid and clot.
- Reduction of neutral red.
- Gas formation in bile salt lactose broth.
- Indol formation in peptone water.
- Ability to grow in phenol broth (·05 per cent.).

As the result of these further tests the number of chemical varieties present became further reduced to 11, and to most of these definite names could be applied, though many differed in one or more reactions from the type form. The following table shows the reactions of these 11 types:—

TABLE VI.—REACTIONS of BACILLI of the COLON GROUP from DRAIN AIR.

Number.	Number of times obtained.	Bile-salt litmus broths.					Voges and Proskauer's reaction.	Motility.	Liquefaction of gelatin.	Gas in gelatin shake culture.	Litmus milk.		Neutral red.	Indol.	Species or variety.
		Lactose.	Saccharose.	Dulcete.	Adonite.	Inulin.					Acid.	Clot.			
1	10 colonies	.	.	.	.	.	.	.	.	.	.	.	.	.	Unidentified. Probably = No. 1 in Table V.
2	4 colonies	+	.	+	.	.	.	?	.	+	+	+	+	+	<i>B. coli</i> communis: typical.
3	1 colony	+	.	.	.	.	.	.	.	+	+	+	+	.	<i>B. coli</i> : atypical.
4	2 colonies	+	+	+	.	.	.	+	.	+	+	+	+	.	<i>B. coli</i> : atypical.
5	2 colonies	+	+	.	+	.	+	.	.	+	+	+	+	.	<i>B. lactis aërogenes</i> : typical.
6	9 colonies	+	+	.	+	.	.	.	.	+	+	+	+	.	<i>B. lactis aërogenes</i> : atypical.
7	1 colony	+	+	.	+	.	.	.	.	+	+	+	+	+	<i>B. lactis aërogenes</i> : atypical.
8	6 colonies	+	+	+	+	.	=	.	.	+	+	+	+	+	Friedländer group.
9	5 colonies	+	+	.	.	.	+	+	+	+	+	+	+	.	<i>B. cloacæ</i> : typical.
10	1 colony	+	+	.	.	.	.	.	?	+	+	+	+	+	<i>B. cloacæ</i> : atypical.
11	1 colony	acid.	+	.	.	.	+	+	.	.	+	+	.	.	<i>B. cloacæ</i> : atypical.

A comparison of these results with those shown in Table V. in which the reactions of the similar organisms most abundant in the local sewage are set forth, will show the very high degree of identity which obtains between the two sets of bacilli. At least six of the types present absolute identity throughout all 13 tests; there can be no doubt that they represent the same organisms, and it is evident that organisms identical with those of sewage were obtained in rich abundance from the air of the drain.

Nevertheless, they were not always found in this abundance. On June 14th I exposed a plate of Conradi and Drigalski's medium, and another of bile salt neutral red lactose agar in the same situation, but for half-an-hour instead of an hour. On this occasion I obtained no *B. coli* or its varieties, though one colony of typical *B. lactis aërogenes* grew, together with a number of colonies corresponding with Type 1, Table VI. On November 22nd, however, an exposure of four Conradi and Drigalski plates in the same place for an hour yielded as many red colonies as on the first occasion, though I did not this time subject the colonies to the full series of tests. It is probable that the conditions under which sewage gives up its bacteria to the air in contact with

it may vary from time to time ; these conditions require more careful study than has yet been bestowed on them.

*Summary of Evidence Derived from Bacilli of the  
Colon Group.*

It is shown by the preceding experiments that from the air of a large drain it is readily possible to isolate members of the *B. coli* group in numbers, and in considerable variety, by the aid of suitable methods. Such bacilli closely correspond, on the whole, in their biological characters with those of the sewage flowing along the drain. From the fresh air in the vicinity these bacilli can only be obtained in extremely scanty numbers.

These facts offer the strongest possible confirmation of the conclusions drawn from the study of the streptococci of sewer and drain air. The bacilli in question must have been derived from the sewage and not from the fresh air.

## SECTION V.

### EVIDENCE AFFORDED BY THE EMPLOYMENT OF A TEST ORGANISM (*BACILLUS PRODIGIOSUS*) AS TO THE LIBERATION OF BACTERIA FROM THE FLUID CONTENTS OF DRAINS.

When the experiments so far detailed were already in large part completed, and when I had independently satisfied myself of the readiness with which sewage organisms could be detected in drain air, I became aware of the experiments conducted by Major Horrocks at Gibraltar (Proc. Royal Soc., 1907, and "Public Health," May 1907). These experiments are even more conclusive than those which I have here brought forward, and they point in the same direction. In some of his most brilliant and convincing observations, Major Horrocks had employed *B. prodigiosus* as a test organism. I therefore carried out on two occasions experiments with *B. prodigiosus*, similar to those recorded by him, with the view of confirming his results. The bacillus in question is eminently suited for a test organism in this connection : its conspicuous red colonies on ordinary agar plates render it easily recognisable, and, though it is not rare in certain soils and waters, I have never met with it in London air or London sewage, though I have made a large number of observations on both during the last ten years. The first of my two experiments was carried out at St. Bartholomew's Hospital : the second in the drains of my own residence at Highgate.

#### EXPERIMENT I.

A control experiment was first carried out to determine again the absence of *B. prodigiosus* from the drain air : an agar plate was exposed at the Little Britain outfall for 30 minutes : no colony of *B. prodigiosus* arose in it.

For the experiment itself I chose an inspection chamber close to one of the ward blocks. The inspection chamber

is some 10½ feet deep and is well ventilated, and provided with an interceptor: into the open channel at the bottom discharge soil pipes from the water-closets of certain of the Hospital wards. The highest of the wards in question is about 60 feet above the ground giving a direct fall of over 70 feet to the bottom of the inspection chamber, so that considerable splashing must arise when the closets are in use. I selected the highest ward in the block for carrying out the experiment. From the inspection chamber the sewage passes along drains gradually increasing in size, and joined by many others, to the 18-inch drain at the Little Britain outfall. The distance from the inspection chamber to the outfall, paced along the devious course of the drain, is 130 yards, and there is a clear air-way for the whole distance—no other interceptor being present along the course of the drain till the outfall itself is reached.

On June 21st a large agar plate was suspended in the inspection chamber by means of a sterilized wire-frame and string: it hung in mid air 4½ feet down the chamber, and 6 feet or more from the bottom, quite clear of the side. The cover was then replaced on the chamber. Four similar agar plates were now exposed at the Little Britain outfall, at distances of 2, 4, 6, and 8 feet from the flowing sewage: the cover of the manhole was then replaced. I had previously prepared a flask of water containing 200 or 300 cc. in which 8 cultures of *B. prodigiosus* were suspended. Having been engaged in making this suspension I regarded myself as a possibly infected person, and I was careful not to touch the plates exposed, or even to descend the drain itself. The plates were exposed and subsequently collected by an independent person, who had not been in contact with the *Bacillus*.

The plates having been exposed I went up to the highest ward in the block and poured the flask of *prodigiosus*-infected water down the water-closet, with repeated flushings: all the the taps in the lavatory were also turned on at the time. An interval of 45 minutes was now allowed to elapse, when the plates were collected, covered, and put in an incubator at 20° C.

*Result.*—After three days, the plate locally exposed in the inspection chamber was found covered with colonies of *B. prodigiosus*: I estimated that some 1,500 colonies of the bacillus were present on the plate: hardly any other colonies were visible. Of the plates exposed at the Little Britain outfall, one showed a single colony of *B. prodigiosus*, namely, the plate exposed furthest from the sewage: the others showed none.

I think that to this single colony, obtained at the Little Britain outfall, much more interest and importance attaches than to the 1,500 colonies found locally in the plate at the inspection chamber itself, where the splashing was gross in character. It is unlikely that the bacillus was carried along 130 yards of airway down the drain, for this would be against the air current which the ventilation of the drainage system was designed to produce, and would, moreover, have been prevented by the local intercepting trap. It is far more probable that the bacillus was disengaged locally from the sewage, either where it gurgles through the interceptor or, 60 feet higher up, where the drain along which it arrived drops a few inches into the main channel.

## EXPERIMENT II.

A similar experiment was carried out in the drainage system of a private house. My own house served well, as the drains were completely known to me, having been relaid throughout 2½ years previously, and being in excellent condition. In order to render the experiment intelligible I must briefly describe the arrangement of the drains.

The house is semi-detached, and all the drains discharge into a main inspection chamber (A), situated at the side of the house, and 11½ feet deep. This chamber receives, in a grooved channel at the bottom, the soil pipes from three water-closets, one in the basement with little fall, one on the ground floor, and one on the first floor. It receives also a 4-inch drain from certain scullery sinks which passes round the back of the house, bending at one point at a right angle. At this point is another inspection chamber (B), 7 feet deep. The distance between this and the main inspection chamber is over 50 feet. The main inspection chamber A is ventilated by a mica valve inlet in a shrubbery, the soil pipes and the scullery drains being carried up to the roof in the usual fashion and serving as outlets. From the main inspection chamber the drains discharge through an intercepting trap into the sewer in the road.

I prepared a broth culture of *B. prodigiosus* in a large flask, and caused half-a-pint of it to be poured into a stoppered bottle; this bottle was then enveloped in sterile cotton wool by an assistant and done up in brown paper. These precautions were adopted in order that I might convey the test organism to my house without any suspicion of personal contamination.

*Control Experiment.*—On the evening of July 13th agar plates were hung in the two inspection chambers A and B by strings about 4 feet long, and the covers of the chambers replaced. A plate was also exposed in the water-closet on the first floor. These three plates were removed next morning, after 13 hours' exposure, and incubated at 20° C. No colony of *B. prodigiosus* appeared in any one of the three.

The experiment itself was carried out during the following night. On the evening of July 14th five agar plates were exposed as follows:—One was hung in the main inspection chamber A, and one in inspection chamber B; one was fixed outside, and touching, the mica valve inlet to inspection chamber A, one was exposed in the ground floor and one in the first floor water-closet. These arrangements having been completed, and the covers of the inspection chambers replaced, the bottle of *prodigiosus* culture was unpacked from its sterilised wrappings and its contents poured, in two successive parts, down the water-closet on the ground floor, with repeated flushings.

On the following morning the plates were collected, covered, and incubated at 20° C. The following results were obtained:—

1. The plate exposed in the main inspection chamber A yielded 92 colonies of *B. prodigiosus*.
2. The plate fixed outside the mica valve inlet yielded no colony of *B. prodigiosus*.

3. The plate exposed in inspection chamber B yielded two colonies of *B. prodigiosus*.
4. Of the plates exposed in the water-closets, the one on the ground floor, where the infecting material had been poured down, yielded one colony of *B. prodigiosus*. The plate upstairs yielded none.

From this experiment the following conclusions may be drawn:—

- (a) In the drainage system of an ordinary private house sufficient splashing occurs to permit of the ready disengagement of bacteria from material passing down soil pipes.
- (b) Such disengaged bacteria may be carried along a 4-inch drain, against the flow of sewage, by the air currents in ventilation, for a distance of at least 50 feet.
- (c) During the actual experiment, the ventilation was acting properly; the inlet was truly an inlet.

*Conclusions from Experiments with B. Prodigiosus.*

The two experiments just related still further confirm the observations contained in Sections III. and IV., as to the readiness with which, under certain conditions, sewage may part with its micro-organisms to the air with which it is in contact. They confirm also, though on a small scale, the results obtained by Major Horrocks at Gibraltar. It is evident that this method of experimenting will prove of much importance in determining many problems in connection with the ventilation of drains and sewers

## SECTION VI.

### GENERAL SUMMARY.

Sufficient evidence has been brought forward in Sections III., IV. and V. of this report to justify the conclusion that under many ordinary circumstances characteristic sewage bacteria are to be found in the air of drains and sewers. The view, till recently current, that sewage does not readily give up its bacteria to sewer air is shown to be incorrect. The evidence is of more than one kind, and it all points in the same direction.

- (1.) In Section III. I have shown that the streptococci of drain air, when tested as to their biological characters, are found to correspond, almost in their entirety, with those of sewage and only to a slight extent with those which chiefly abound in fresh air. The types of streptococci which are most abundant in fresh air were not found in the air of sewers and drains. The evidence obtained from the streptococci of drain air in the City of London is in harmony with that previously obtained from the streptococci of the air of main sewers in Hampstead.
- (2.) In Section IV. I have shown that bacilli of the colon group obtained from drain air, correspond essentially in their biological characters with those of sewage, and that such bacilli can only be obtained from fresh air

with great difficulty or not at all. The evidence obtained under this section from drain air in the City of London is far more convincing than that which I was able to obtain previously from the sewer air of Hampstead.

- (3.) Lastly, it is shown that a test microbe, added in bulk to the contents of the drains under suitable conditions of splashing, can be recovered locally from the drain air in great numbers, and can also be recovered from the air at considerable distances (50 feet and 390 feet) from the point at which it was added to the sewage.

From this evidence, no less than from that obtained by Major Horrocks, it may be taken as conclusively proved that under certain circumstances, at all events, sewage gives up its bacteria to sewer and drain air. Such bacteria may form but a small proportion of those present in sewer air: this is likely, for they would not otherwise have escaped detection by previous observers. But it is probable that the circumstances under which sewage gives up its bacteria are common and ordinary circumstances in sewer and drain construction, for the employment of selective culture media has enabled me to discover them wherever so far looked for in the air of drains and sewers.

It is evident that a closer study of the numerical abundance of characteristic sewage organisms, in sewer and drain air, at different points in a drainage system, and under varying conditions of flow of sewage, will throw light on the more precise circumstances under which sewer air becomes polluted by sewage microbes, and to this task I hope to devote myself as the next step in the inquiry.

The importance of the subject is plain, for though the organisms which I have been able to detect in sewer air are not in themselves known to be prejudicial to health, being for the most part well-known saprophytes of the normal alimentary tract, yet their value is evident as *indices* of the possible presence of more harmful sewage-borne microbes.

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## APPENDIX B., No. 4.

## REPORT on BACTERIAL STUDIES of MILK from HEALTHY and DISEASED COWS; by WILLIAM G. SAVAGE, M.D.

Milk is a fluid which has frequently been shown to be a vehicle by means of which disease has been transmitted. Speaking broadly, such disease-producing contamination may have one of the three following sources of origin :—

- (a.) The infective matter may be derived from the cow supplying the milk.
- (b.) The infective matter may obtain access to the milk either from those handling it being themselves in a diseased condition or from their acting as carriers of infection.
- (c.) The infective matter may be derived from manurial matters or other filth materials which have gained access to the milk at the time of milking, in transit, or at some other period during its passage from the cow to the consumer.

Illustrations of these different methods of contamination readily present themselves.

The present inquiry is entirely concerned with the possibility of the cow serving as the source of infective matters found in the milk.

Apart from tuberculosis there is considerable difference of opinion as to how far, and to what extent, milk may be rendered infective through altered conditions of the cow or locally of the udder.

The most convincing evidence in this connection is that associating diseased conditions of the cow's udder with outbreaks of sore throat.

This connection has invariably been established, at least in its more convincing aspects, upon epidemiological grounds, and, as far as I am aware, in no case has complete bacteriological proof been supplied.

Certain specific elements have been directly credited with a disease-producing rôle. In particular, streptococci derived from milk have been associated with the causation of gastro-intestinal diseases and with sore throat. So also all purulent conditions of the udder and teats have been suspected of disease causation properties.

On the other hand, such a connection is by no means admitted by many veterinary authorities, and the evidence at present available must be admitted to be circumstantial and cumulative rather than precise.

Accurate investigations are very desirable to ascertain—

- (1.) How far streptococci and pus cocci generally are present in milk from healthy cows and the relationship of such organisms to morphologically similar pathogenic bacteria.
- (2.) To what extent leucocytes are present in milk, and how far their presence is determined by a purulent or inflamed condition of the udder and with the presence of pyogenic organisms,

- (3.) The bacteria associated with inflamed and ulcerated conditions of the cow's udder and how far they may be ascribed a disease producing rôle for human beings.

The present inquiry is divided into two parts. Part I. is concerned with these two first considerations, while Part II. includes some preliminary work upon the bacterial content of inflamed udders.

## PART I.

### THE MILK OF APPARENTLY HEALTHY COWS WITH PARTICULAR REFERENCE TO THE PRESENCE OF LEUCOCYTES AND OF STREPTOCOCCI.

#### SECTION I.

##### *Methods adopted.*

A. *Collection of samples.*—All the milk samples, unless otherwise indicated, were from reputedly healthy cows, in ordinary country cowsheds, yielding milk which was being mixed with that of other cows, and either vended in the ordinary way or consumed by the owner's family.

The samples were all obtained from individual cows, collected at the afternoon milking time under my personal supervision. The examination was in every case started within two hours of their collection.

The samples were collected into sterile narrow-mouth 2-oz. glass stoppered bottles, the milk being milked direct into them, care being taken that the teat did not touch the bottle.

During collection the stopper was held so that the part going into the bottle did not touch anything, and was replaced immediately after collection.

Unless otherwise mentioned, all the samples were from the middle milk, the fore milk being milked into the pail from each quarter before the sample was collected.

Unless otherwise stated, each sample was a mixture of the milk of all four quarters in approximately equal amount.

The udders and teats were in every case clean to the naked eye, but no cleaning or antiseptic precautions were used.

The milker's hands, apart from ordinary washing, were not specially cleansed, except in a few instances.

B. *Determination of the presence of streptococci and tests used for their differentiation.*—This was arrived at in several different ways :—

- (a.) Varying quantities of the milk were added by sterile pipette to tubes of nutrient glucose broth or neutral-red glucose broth. These were incubated for two days at 37° C., and then the presence or absence of streptococci chains was ascertained by examining a little of the fluid in hanging drop preparations. A positive result was recorded only when quite definite chains of cocci were detected, or in doubtful cases when stained preparations showed such definite chains. 0.1 and 1.0 cc. were the amounts usually added to broth in this way. In this method merely the presence or absence of streptococci was noted.

- (b.) 10 cc. of the milk were centrifugalised in a sterile centrifugal tube (running about 1,800 revolutions per minute) for 10 minutes. Either all or a considerable part of the deposit so obtained was then transferred to a Petri dish containing solidified nutrient agar, and the deposit was distributed evenly over the surface by means of a sterile glass rod. As a rule only one agar plate was used, but sometimes two or more were employed. The plates were incubated for one or two days at 37° C. The possible streptococcus colonies were first isolated in broth or upon agar slope media, and if microscopic examination showed them to be streptococci, their characters were more completely determined.
- (c.) The stained deposit of a fresh 10 cc. of the milk used for the differential leucocyte enumeration (as described below) was carefully examined for the presence of streptococci or other bacteria.

The individual streptococci were all isolated from the agar plates. The cultural characters relied upon for their differentiation were: Growth upon sloped nutrient agar; growth in nutrient broth; growth upon sloped nutrient (12 per cent.) gelatin; action upon litmus milk; the production of acid in the sugar and alcohol media introduced by Gordon, *i.e.*, action upon lactose, saccharose, salicin, mannite, coniferin, raffinose, and indol. The determination whether reduction of neutral red occurs under anaërobic conditions was not made.

The other characters investigated were the morphological characteristics when grown in liquid media, and, for a number of the streptococci, a determination of pathogenicity to mice.

All the streptococci which were tested stained by Gram's method.

A reaction of + 1 per cent. was found most suitable for both the broth and agar media, while nutrient glucose (1 per cent.) broth was found preferable to simple nutrient broth.

Incidentally the presence of staphylococci was also noted in many instances.

*C. Determination of the presence of B. coli and allied glucose fermenting organisms.*—10 cc. of the milk were added by sterile pipette to a sterile empty test tube and incubated at 37° C. for 24 to 48 hours. Three platinum loopfuls were then added to a tube of glucose bile-salt peptone, which was incubated for two days at 37° C. If no gas developed in the inner tube, glucose fermenters were assumed to be absent in the 10 cc. of milk. If gas developed, the fermenting organism was isolated upon suitable solid media and its characters determined. For most of the samples the presence, or absence, of glucose fermenters in 1 cc. was also investigated by the addition of that quantity of the milk to glucose neutral red broth, in a double tube, and plating if gas developed.

*D. Determination of the number of leucocytes in milk.*—As a rule the number of leucocytes is usually judged from the number present in stained films made from the sedimented milk. This is a very inaccurate and unreliable procedure, the results of which vary greatly according to the thickness of the film.

A special method for estimating the number of leucocytes was employed. This method has been previously described by me in the "Journal of Hygiene" (Vol. VI., p. 123, April, 1906), but is briefly reproduced here for convenience of reference and for the proper understanding of the way the count is arrived at.

The ordinary Thoma-Zeiss blood-counting chamber, in use in most laboratories, is employed. Direct counting of the leucocytes is impossible owing to the opacity caused by the large amount of fat.

One cc. of the milk is accurately transferred to a special centrifugal tube (see Figure 1), and freshly filtered Toisson's solution\* is poured in to almost fill the tube, which has a capacity of about 15 cc.

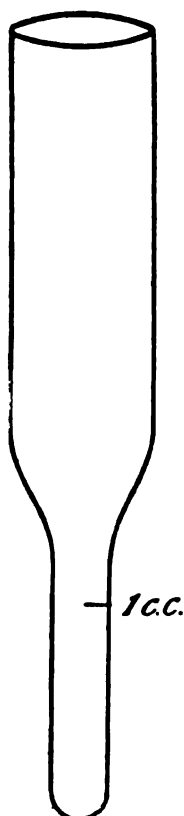


FIG. 1.

The two fluids are well mixed and then centrifuged for ten minutes. The machine used was an electrically driven centrifuge, giving about 1,800 revolutions per minute.

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\* This is the well-known indifferent solution used in blood enumerations. It does not injure the leucocytes, but stains them enough to render them clearly visible. Its composition is methyl violet 0.025 grams, sodium chloride 1 gram, sodium sulphate 8 grams, glycerine 30 cc., distilled water 160 cc.

The cream is then well broken up by a clean glass rod, to disentangle any leucocytes carried to the surface, and the mixture is centrifugalised for an additional 5 minutes.

All the fluid is then removed except the last 1 cc., great care being taken not to disturb the deposit.

This can be conveniently and readily done by means of a fine glass tube connected to an exhaust pump.

Theoretically all the leucocytes present in the original 1 cc. of milk are now present in the 1 cc. of fluid, the object of the above manipulation being solely to get rid of the fat. The following calculation is based upon such an assumption.

The leucocytes are now thoroughly well mixed and distributed through the 1 cc. A sufficient quantity is then placed on the ruled squares of the Thoma-Zeiss apparatus, the cover glass put on, and the preparation examined exactly in the same way as for the enumeration of the white corpuscles in blood.

If they are very numerous the leucocytes can be counted on the squares, but nearly always the counting must be done by fields. To do this the tube of the microscope is drawn out until an exact number of squares spans a diameter of the field of vision. Then the leucocytes in a number of different fields of vision are counted moving regularly from one field of vision to another. Twenty different fields of vision were almost invariably counted, occasionally 30 or more.

The number of leucocytes in all the fields counted, divided by the number of fields counted, gives, of course, the average number per field.

The number of leucocytes per cubic mm. of milk =  $\frac{56,000y}{11d^2}$

where  $y$  = the average number of leucocytes per field of vision, and  $d$  = the number of squares which just spans the diameter,  $d$  is determined once for all by marking the draw tube, so that only 20 fields have to be counted and the figures substituted in the formula. In practice the method has been found simple and reliable.

With practice leucocyte enumerations can be performed without taking up an undue amount of time.

*E. Numerical determination of the different kinds of leucocytes in milk.*—In the slightly stained preparations used in the Thoma-Zeiss counting chamber for the numerical estimation of the leucocytes, several varieties of leucocytes could readily be differentiated, and for the earlier samples an attempt was made to ascertain the relative and actual proportion of these different kinds. No results of value were, however, obtained, and they are therefore not included in the report.

In every instance the centrifugalised deposits were stained and examined. For this purpose 10 cc. of the milk were centrifugalised for 10 minutes. The cream and all except the deposit was poured off, the tube (capacity 20 cc.) filled with normal saline solution, and the contents again centrifugalised for five minutes. The fluid was then poured off from the firm deposit and the latter spread over coverslips, dried, fixed and stained, in Löffler's methylene blue, for five minutes or longer.

For films prepared as above no advantage was found to be gained by soaking in ether before staining.

TABLE 1.

Number of cow.	Number of calves.	Period since last calv.	Time to next parturition.	History of previous udder inflammation or injury.	Number of leucocytes per cubic mm. of milk.	Presence of streptococci in		Amount of milk yielded.	Udder and teat condition.
						0.1.	1.0 cc.		
1	—	—	Not pregnant	Nil ...	285	—	+	Abundant	Nil abnormal.
2	3	—	3 months	Nil ...	3,610	—	+	Almost nil	Nil abnormal.
3	—	—	3 months	Nil ...	1,280	+	+	A few pints	A lump in left hind quarter.
4	1	6 months	Not pregnant	Nil ...	50	—	—	Abundant	Nil abnormal.
5	3 or more.	3 weeks	Not pregnant	Nil ...	3,320	—	+	Abundant	One teat tender. Milk distinctly light brown in colour.
6	—	3 weeks	Not pregnant	Nil ...	210	—	+	Abundant	Nil abnormal.
7	—	4 weeks	Not pregnant	Nil ...	80	—	+	Abundant	Nil abnormal.
8	More than 6.	7 weeks	Not pregnant	Nil ...	270	+	+	Abundant	Nil abnormal.
9	3	3 weeks	Not pregnant	Nil ...	106	+	+	Abundant	Nil abnormal.
10	6	—	Not pregnant	Nil ...	180	—	—	Abundant	Nil abnormal.
11	1	3 months	Not pregnant	Nil ...	540	—	—	Abundant	Nil abnormal.
12	1	2 weeks	Not pregnant	Nil ...	150	—	+	Abundant	Nil abnormal.
13	1	—	4 months	Nil ...	230	—	+	Abundant	Nil abnormal.
14	—	5 months	Not pregnant	R. H. Q. injured 3 months previously.	2,010	+	+	Abundant	Nil abnormal.
15	More than 8.	2½ months	Not pregnant	Nil ...	50	—	—	Abundant	Nil abnormal.
16	More than 8.	6 weeks	Not pregnant	Nil ...	85	—	—	Abundant	Nil abnormal.
17	4	4 months	Not pregnant	Nil ...	330	+	+	Abundant	Nil abnormal.
18	2	2½ weeks	Not pregnant	Nil ...	520	—	—	Abundant	Nil abnormal.
19	2	—	2 months	Nil ...	230	+	+	Diminishing. Milked once a day only.	Right hind teat ulcerated.
20	More than 2.	2 months	Not pregnant	Nil ...	2,650	+	+	Abundant	Nil abnormal.
21	4	2 weeks	Not pregnant	Nil ...	3,700	—	+	Abundant	Nodule, size of a walnut at base of left fore teat and left hind teat. Not tender.

23	1	3 months	Not pregnant	Nil ...	...	28	+	Abundant	...	Nil abnormal.
24	—	4 weeks	Not pregnant	Nil ...	...	50	+	Abundant	...	Nil abnormal.
25	2	3 months	Not pregnant	Nil ...	...	85	+	Abundant	...	Nil abnormal.
26	3	—	4 months	L. H. Q. about a year previously.	1,600	+	+	Abundant	...	Nil abnormal.
27	3	—	3 months	Nil ...	270	+	+	Abundant	...	Nil abnormal.
28	4	3 months	Not pregnant	Nil ...	490	+	+	Abundant	...	Nil abnormal.
29	1	—	3 months	Nil ...	560	+	+	Diminishing	...	Nil abnormal.
30	3	—	2 months	Nil ...	940	+	+	About 1/2 gallon a day only.	...	Nil abnormal.
31	1	—	3 months	Nil ...	130	+	+	Starting to diminish	...	Nil abnormal.
32	1	—	2 months	Nil ...	56	+	+	Abundant	...	Nil abnormal.
33	3	3 weeks	—	Nil ...	200	+	+	Abundant	...	Nil abnormal.
34	4	—	4 months	Nil ...	105	+	+	Abundant	...	One test ulcerated.
35	1	3 months	—	Nil ...	670	+	+	Abundant	...	Nil abnormal.
36	1	—	4 months	Nil ...	84	+	+	Abundant	...	Nil abnormal.
37	2	—	7 weeks	Nil ...	660	+	+	6-8 pints only	...	Nil abnormal.
38	2	—	8 weeks	Nil ...	490	+	+	8-10 pints only	...	Nil abnormal.
39	2	—	2 months	Nil ...	4,100	+	+	Only 2 or 3 pints a day. Only milked once a day.	...	Nil abnormal.
40	4	6 weeks	Not pregnant	Nil ...	200	+	+	Abundant	...	Nil abnormal.
41	2	4 days	Not pregnant	Nil ...	250	+	+	Abundant	...	Nil abnormal.
42	3	5 weeks	Not pregnant	Nil ...	460	+	+	Abundant	...	Nil abnormal.
43	3	6 weeks	Not pregnant	Nil ...	85	+	+	Abundant	...	Nil abnormal.
44	3	6 weeks	Not pregnant	Nil ...	295	+	+	Abundant	...	Nil abnormal.
45	4	3 1/2 months	Not pregnant	Nil ...	100	+	+	Abundant	...	Nil abnormal.
46	1	—	3 months	Nil ...	64	+	+	Abundant	...	Nil abnormal.
47	5	5 months	Not pregnant	Milk fever 5 months previously.	1,025	+	+	Abundant	...	Nil abnormal.
48	3	4 months	—	Nil ...	270	+	+	Abundant	...	Nil abnormal.
49	2	32 hours	Not pregnant	Nil ...	1,265	+	+	Abundant	...	Tests tense and hot.
50	2	4-5 months	—	Nil ...	14	+	+	Abundant	...	Udder normal. Extensive test ulceration.
51	1	3 weeks	Not pregnant	Nil ...	85	+	+	Abundant	...	Nil abnormal.
38A	Cow 38,	re-examined	2 weeks later	Nil ...	132	+	+	Abundant	...	Nil abnormal. Cow 26 examined
26A	4	3 days	Not pregnant	Nil ...	320	+	+	Abundant	...	38 days later, after premature birth.

A number of different stains, including the well-known stains of Jenner and Leishman, were tried for differentiating the leucocytes, but on the whole the results obtained by simple staining with methylene blue, as above described, were the most satisfactory.

I am greatly indebted to Alderman Wilson Marriage, Dilbridge Hall, Colchester, for his kindness in allowing me to make free use of his extensive herd of cows for purposes of examination, and for facilitating the examination.

Table 1 contains some general particulars as to the number of calves, period since last parturition, stage of pregnancy, etc. In each case the existence of previous udder inflammation and udder injury was inquired into. An examination of the udder and teats of each cow was made and the results are recorded in the table.

The leucocyte count and the presence or absence of detected streptococci in 0.1 and 1.0 cc. of the milk, when added to broth, incubated at 37° C. for two days and then examined in hanging drop preparation, are also recorded.

## SECTION II.

### STREPTOCOCCI IN THE MILK OF INDIVIDUAL COWS.

Streptococci have been proved to be the causal agents of a number of diseases, while with good reason they are suspected of being the cause of others, although the proof is less complete.

On the other hand streptococci as a group are saprophytic in the buccal cavity and intestine of the animal body. More intimately concerned with milk problems, streptococci are associated with at least some cases of mastitis of the cow and goat, while they have been, as already mentioned, suspected of causing outbreaks of diarrhoea and sore throat, the medium of conveyances being milk.

It is obviously, therefore, of great importance to consider whether streptococci are present only in milk from diseased or unhealthy animals, or whether they are met with in milk as obtained direct from the healthy cow and without chance of outside pollution.

It is of equal importance to consider the actual kinds present, and how far they are identical with those associated with pathological processes in man, and if the varieties found bear any relationship to diseased or abnormal conditions of the udder or teats of the cow in the milk of which they are found to be present.

*A. The general presence of streptococci in milk.*—An examination of ordinary mixed milk shows the presence of streptococci in large numbers. Undoubtedly their great abundance is in part to be explained by their rapid multiplication in milk. On the other hand, mixed milk samples collected immediately after milking show numerous streptococci. No mixed milk samples were examined for this report, but in a previous investigation, reported in the paper in the "Journal of Hygiene" already

referred to, in 11 samples of milk collected quite fresh at the farm and examined within three hours, streptococci were found in all.

In 80 per cent. streptococci were found in 0.1 cc., and in 100 per cent. in 1.0 cc.

The results obtained with samples derived from individual cows throw some light upon the source whence these streptococci gain access to the milk.

Three separate series of results were obtained, according to the procedure adopted.

*a. The presence of streptococci as shown in broth preparations.*—Table 1 shows how far streptococci, as such, were found to be present in broth cultures to which varying quantities of milk had been added and then incubated at 37° C for 40–48 hours.

From this table it will be seen that streptococci were present in the majority of the samples. Taking the 1.0 cc. figures; out of 44 examinations in which 1 cc. of the middle milk was added to broth; in 29, or 66 per cent., the presence of streptococci could be definitely made out.

When smaller quantities of the milk were examined, *e.g.*, 0.1 cc., streptococci were present in 11 out of 30 examined, or 37 per cent.

*β. The presence of streptococci as shown in stained preparations made from the centrifugalised deposit.*—In every instance the stained deposit of 10 cc. of milk was carefully examined for the presence of chains of cocci and for bacteria generally.

On a few occasions diplococci were met with, but only in one instance were cocci in chains found, *i.e.*, in the deposit of milk from No. 42.

All the stained films were carefully examined to see if organisms with the morphological appearances of the diplococcus intracellularis meningitidis were present, but no cocci in any way resembling that organism were met with.

*γ. The presence of streptococci as shown by their isolation from the centrifugalised deposit.*—In all, except the first 12 samples, and for two other samples, the deposit of 10 cc. of the milk, centrifugalised for 10 minutes, was brushed over one or more agar plates.

As a rule a large number of colonies developed, and it was not found possible, in most cases, without sub-cultivation to be certain which colonies were those of streptococci. The large ordinary staphylococcus colonies could be readily distinguished, but a peculiar type of coccus, which grew either not at all or with great difficulty in broth, was very frequently present, the colonies of which upon agar are minute and circular, although generally less translucent than those of streptococci. Frequently, although a large number of possible colonies were sub-cultivated either into broth or on to agar slope (usually the latter), none of them turned out to be streptococci. The inability to isolate streptococci from these agar plates cannot be taken as definitely showing that they were absent in the amount of milk examined, but it does show that if present they were only in very scanty numbers.

Out of 38 milk deposits so plated, in only 18 (47 per cent.) were streptococci isolated.

This is sufficient to explain the considerable difference shown between these results and those obtained by incubating the broth media to which milk has been added.

In Table 2 a brief summary is given of the different kinds of colonies present on the agar plates brushed from the centrifugalsed deposits.

TABLE 2.

Number of cows.	Notes on the different colonies present on the agar plates.	Organisms sub-cultivated and investigated.	
		Streptococci (Table 3).	Staphylococci (Tables 4 and 5).
1 to 12	(Inclusive.) Centrifugalsed deposit not plated		
13	Numerous staphylococci, also typical streptococci	16, 17, 18	
14	Some staphylococci. Diphtheroid B. colonies very numerous. No streptococci isolated.		1, 39
15	Very numerous diphtheroid B. colonies. Also ordinary staphylococcus and translucent staphylococcus colonies. No streptococcus colonies found.		2, 40, 41
16	A few staphylococci only ... ..		
17	Many thousands of minute colonies apparently all streptococci as proved by sub-cultivation. Also some staphylococcus, but no diphtheroid colonies.	19, 20, 21, 22, 23.	42, 43
18	Only a few colonies. No streptococci ... ..		
19	Staphylococcus and streptococcus colonies both very numerous.	27, 28, 29	44
20	Staphylococcus and streptococcus colonies both very numerous.	30, 31, 32, 33, 34.	
21	Only a few colonies, nearly all staphylococci. One diphtheroid bacillus colony.	35, 36	45
22	Ordinary staphylococcus and translucent staphylococcus colonies numerous. No streptococci found, all the minute colonies sub-cultivated being translucent staphylococci.		3, 4, 5, 6
23	Very similar to No. 22 ... ..		
24	Very numerous ordinary staphylococci, and a few translucent staphylococci. No streptococci.		7, 8, 46, 47, 9, 10, 11
25	Numerous ordinary staphylococci. Two streptococci isolated. The vast majority of the fairly numerous minute colonies are translucent staphylococci.	37, 38	12, 13, 14, 15.
26	Numerous white and yellow staphylococcus and translucent staphylococcus colonies. No streptococci.		16, 17, 50
27	Many hundreds of colonies, some streptococci, some diphtheroid B., some translucent staphylococci, some ordinary staphylococci. The great majority are staphylococci, and streptococci only found with difficulty.	39, 40	45, 48
28	Very numerous colonies. The vast majority are ordinary white staphylococci. No streptococci found.		51, 52

TABLE 2—continued.

Number of cow.	Notes on the different colonies present on the agar plates.	Organisms sub-cultivated and investigated.	
		Strepto- cocci (Table 3).	Staphylo- cocci (Tables 4 and 5).
29	Very numerous colonies both ordinary staphylococci and streptococci. Streptococci readily isolated.	41, 42, 43 44, 45, 46.	
30	Not plated ... ..		
31	Numerous ordinary staphylococci and a good many translucent staphylococci. No streptococcus colonies.		18, 19, 20
32	Similar to No. 31 ... ..		21, 22
33	Many thousands of minute colonies all streptococci. Only a few staphylococci.	47, 48, 49 50, 51, 52.	
34	Not plated ... ..		
35	A few ordinary and numerous translucent staphylococcus colonies. No streptococci.		23, 24, 25
36	Numerous ordinary and a few minute translucent staphylococcus colonies. No streptococci.		
37	Similar to No. 36 ... ..		
38	Ordinary and translucent staphylococci both very numerous. No typical streptococcus colonies, and no streptococci to be isolated.		26, 27, 28
39	Both kinds of staphylococcus colony numerous. One streptococcus colony found.	58	29
40	Very few colonies. One streptococcus only found	59	
41	Both kinds of staphylococcus colony numerous. All the minute colonies sub-cultivated were translucent staphylococci.		
42	Very numerous streptococcus colonies. Ordinary staphylococci colonies also fairly numerous.	60, 61, 62 63.	
43	Numerous staphylococci and some streptococci.	64, 65	
44	Both kinds of staphylococcus colonies only.		
45	Both kinds of staphylococcus colonies very numerous. No streptococcus colonies to be found.		
46	Similar to No. 39. (The streptococcus isolated died out.)		
47	Both kinds of staphylococcus colonies only.		
48	Only a few colonies. All staphylococcus.		
49	Both kinds of staphylococcus and some streptococcus colonies.		
50	Numerous staphylococcus and some streptococcus colonies.		
33A	About 50 colonies, half being ordinary staphylococcus and half streptococcus colonies.	53, 54, 55	
26A	Both kinds of staphylococcus colonies numerous. Also some streptococcus colonies.	56, 57	30

Table 2 shows that staphylococcus colonies were present in the great majority of the deposits plated, and as a rule were present in large numbers.

There were two distinct classes of staphylococcus met with, and as a rule both classes were present.

Streptococcus colonies were much less frequently met with, and in only seven cases were they very abundantly met with, *i.e.*, cows 17, 19, 20, 29, 33, 42, 33A.

Bacilli as a class were only occasionally met with, and usually in quite scanty numbers.

B. *The characters of the streptococci, staphylococci and other organisms met with in milk from individual cows.*—Chief attention was given to the streptococci, and only a comparatively small number of staphylococci were culturally investigated.

(1.) *Streptococci.*—The characters of 71 streptococci isolated from these presumably healthy cows is given in Table 3. This table shows that a great many different varieties were present.

As regards their morphology, chains of every length were met with. The length of chain was determined from an examination of *liquid* cultures in hanging drop preparations.

Twelve were short chains, 12 short and medium, 15 long and medium, and 32 long and very long.

Classified into short (including short and medium) and long (including long and medium), a more convenient and stable classification, 24 were short, *i.e.*, 33·8 per cent., and 47 were long, *i.e.*, 66·2 per cent.

When, however, only different streptococci are included from the same cow, only 45 streptococci have to be considered. Of these 42 per cent. were short, and 58 per cent. long chain forms.

TABLE 3.

Number.	Source.	Morphology (length of chains).	Character of broth growth.	Acid in 3 days in						Coagulation of milk.	Pathogenicity.
				Lactose.	Saccharose.	Mannite.	Salicin.	Coniferin.	Raffinose.	Inulin.	
1	Cow 1	Short and medium	Turbid .. ..	+	+	-	+	+	+	+	-
2	Cow 1	Short and medium	Turbid .. ..	+	+	-	+	+	-	-	-
3	Cow 5	Long .. ..	Turbid .. ..	+	+	-	+	+	-	-	+
4	Cow 6	Very long .. .	Clear: marked deposit.	+	+	-	+	+	-	-	+
5	Cow 8	Medium and long	Uniform turbidity	+	-	-	-	-	+	+	+
6	Cow 8	Long .. ..	Clear: deposit ..	+	-	+	+	+	+	-	+
7	Cow 8	Very long .. .	Clear: deposit ..	+	+	-	-	+	-	-	+
8	Cow 8	Short and medium	Uniform turbidity	+	+	-	+	+	-	-	+
9	Cow 9	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	+
10	Cow 9	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	+
11	Cow 9	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	+
12	Cow 9	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	+
13	Cow 12	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	-

TABLE 3—continued.

Number.	Source.	Morphology (length of chains).	Character of broth growth.	Acid in 3 days in						Coagulation of milk.	Pathogenicity.
				Lactose.	Saccharose.	Mannite.	Salicin.	Coniferin.	Raffinose.	Inulin.	
14	Cow 12	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	-
15	Cow 12	Short.. ..	Uniform turbidity	+	+	+	+	+	-	-	-
16	Cow 13	Short and medium	Uniform turbidity	+	+	-	+	-	+	-	+
17	Cow 13	Short and medium	Uniform turbidity	+	+	-	-	-	+	-	+
18	Cow 13	Medium and long	Uniform turbidity	+	+	-	+	-	+	-	+
19	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	-	+	-	+	-	+
20	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	-	+	-	+	-	+
21	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	-	+	-	+	-	+
22	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	-	-	-	+	-	+
23	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	+	+	+	+	-	+
24	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	-	+	-	+	-	+
25	Cow 17	Long .. ..	Nearly clear: marked deposit.	+	+	-	+	-	+	-	+
26	Cow 17	Short and medium	Uniform turbidity	+	+	-	+	-	+	-	+
27	Cow 19	Long .. ..	Clear: marked de- posit.	+	+	-	+	-	+	-	+
28	Cow 19	Long .. ..	Clear: marked de- posit.	+	+	-	+	-	+	-	+
29	Cow 19	Long .. ..	Clear: marked de- posit.	+	+	-	+	+	+	-	+
30	Cow 20	Short.. ..	Uniform turbidity	+	+	+	+	+	-	+	+
31	Cow 20	Medium and short	Clear: slight de- posit.	+	+	-	+	-	-	-	+
32	Cow 20	Short.. ..	Uniform turbidity	+	+	-	+	-	-	+	+
33	Cow 20	Short.. ..	Uniform turbidity	+	+	+	+	-	-	+	-
34	Cow 21	Short.. ..	Uniform turbidity	+	+	+	+	+	-	+	-
35	Cow 21	Very long ..	Clear: marked de- posit.	+	+	-	+	-	+	-	+
36	Cow 21	Very long ..	Clear: marked de- posit.	+	+	-	+	-	+	-	+
37	Cow 25	Long .. ..	Clear .. ..	+	+	+	+	+	-	+	-
38	Cow 25	Short: conglomer- ate.	Clear .. ..	+	+	-	+	+	+	-	-
39	Cow 27	Medium and long: much interlaced.	Clear: marked de- posit.	+	+	+	+	+	+	+	+
40	Cow 27	Medium and long: much interlaced.	Clear: marked de- posit.	+	+	+	+	+	+	+	+
41	Cow 29	Medium and long	Slight turbidity: deposit.	+	+	-	+	+	+	-	+
42	Cow 29	Long .. ..	Slight turbidity: deposit.	+	+	-	+	+	+	-	+
43	Cow 29	Long .. ..	Slight turbidity: deposit.	+	+	-	+	+	+	-	+
44	Cow 29	Long .. ..	No growth.. ..	+	+	-	-	+	+	+	+
45	Cow 29	Long .. ..	Slight turbidity: deposit.	+	+	-	+	+	+	+	+
46	Cow 29	Long .. ..	Slight turbidity: deposit.	+	+	-	+	+	+	-	+
47	Cow 33	Very long ..	Clear: marked de- posit.	+	+	-	-	-	+	-	+
48	Cow 33	Very long ..	Clear: marked de- posit.	+	+	-	+	-	+	-	+

TABLE 3—continued.

Number.	Source.	Morphology (length of chains).	Character of broth growth.	Acid in 3 days in							of Coagulation milk.	Pathogenicity.
				Lactose.	Saccharose.	Mannite.	Salicin.	Coniferin.	Raffinose.	Inulin.		
49	Cow 33	Very long .. ..	Clear: marked deposit.	+	+	-	+	-	+	-	+	-
50	Cow 33	Very long .. ..	Clear: marked deposit.	+	+	-	+	-	+	-	+	-
51	Cow 33	Very long .. ..	Clear: marked deposit.	+	+	-	+	-	+	-	+	-
52	Cow 33	Very long .. ..	Clear: marked deposit.	+	+	-	+	-	+	-	+	-
53	Cow 33A	Medium and long: much interlaced.	Nearly clear: marked deposit.	+	+	-	+	-	-	+	+	-
54	Cow 33A	Medium and long: much interlaced.	Nearly clear: marked deposit.	+	+	-	+	-	-	+	+	-
55	Cow 33A	Medium and long: much interlaced.	Nearly clear: marked deposit.	+	+	-	+	-	-	+	+	-
56	Cow 26A	Very long .. ..	Uniformly turbid: marked deposit.	+	+	+	+	+	+	+	-	-
57	Cow 26A	Very long .. ..	Uniformly turbid: marked deposit.	+	+	+	+	+	+	+	-	-
58	Cow 39	Quite short . . .	Clear: marked deposit.	+	+	+	+	-	+	+	-	-
59	Cow 40	Quite short.. ..	Uniform turbidity	+	+	+	+	+	+	-	-	-
60	Cow 42	Medium and long	Slight turbidity: marked deposit.	+	+	-	-	-	-	-	+	-
61	Cow 42	Medium and long	Slight turbidity: marked deposit.	+	+	-	-	-	-	-	+	-
62	Cow 42	Medium and long	Slight turbidity: marked deposit.	+	+	-	+	-	-	-	+	-
63	Cow 42	Medium and long	Turbid: marked deposit.	+	+	-	+	-	-	-	+	-
64	Cow 43	Medium and long	Turbid: marked deposit.	+	+	-	+	-	-	-	+	-
65	Cow 43	Medium and long	Turbid: marked deposit.	+	+	-	+	-	-	-	+	-
66	Cow 49 R. F. Q.	Long .. ..	Clear: coherent abundant deposit.	+	+	-	-	-	+	-	+	-
67	Cow 49 R. F. Q.	Long .. ..	Clear: coherent abundant deposit.	+	+	-	-	-	+	-	+	-
68	Cow 49 R. F. Q.	Short and medium	Slight turbidity: deposit.	+	-	-	-	-	-	-	+	-
69	Cow 49 R. F. Q.	Short and medium	Slight turbidity: deposit.	+	+	+	+	-	+	+	+	-
70	Cow 49 R. F. Q.	Medium and long	Slight turbidity: deposit.	+	+	+	+	-	+	+	+	-
71	Cow 49 R. H. Q.	Short and medium	Clear: abundant deposit.	+	-	+	-	-	-	-	-	-

The action upon the different sugar-alcohol media shows that the streptococci isolated belong to a number of different varieties.

71 or 100 per cent. fermented lactose.

67 or 94 per cent. fermented saccharose.

22 or 31 per cent. fermented mannite.

59 or 83 per cent. fermented salicin.

31 or 44 per cent. fermented coniferin.

42 or 59 per cent. fermented raffinose.

19 or 27 per cent. fermented inulin.

57 or 80 per cent. coagulated milk.

These figures although they deal with only 71 streptococci may be compared with results obtained by Houston and reported in

these Reports, 1904-5, p. 371. Houston gives the following table, to which I have added my results in the last column :—

—	Cow dung.	Human fæces.	Milk.	Individual healthy cows.
	Per cent. +	Per cent. +	Per cent. +	Per cent. +
Salicin test ... ..	93	92·67	60	83
Saccharose test ... ..	89	86·34	90	94
Lactose test ... ..	85	76·34	97	100
Litmus milk test... ..	73	61·67	70	80
Neutral red broth test ...	All negative	39·34	20	—
Raffinose test ... ..	74	32	19	59
Mannite test ... ..	All negative	24·34	20	31
Inulin test ... ..	13	4·67	21	27
Coniferin test ... ..	—	—	—	44

This table shows that the results obtained with milk samples from individual cows approximate more closely to those obtained with mixed milk samples than to the cow-dung or human fæces results. The chief differences are in regard to the fermentation with salicin and raffinose.

It is of great interest that Houston found that almost all (97 per cent.) the streptococci from cow dung formed short chains in broth with turbidity of the medium.

The streptococci from individual cows differ, therefore, extensively from those of cow-dung, both in regard to their morphology and their action upon the different sugar alcohols. This fact is of interest as showing that the source of many of these udder streptococci is either other than from cow-dung, or that if so derived their sojourn in the udder has altered their biological characters to a considerable extent.

Classified according to their powers of fermentation they form a large number of different groups.

The most frequently present group is a long chain form which ferments lactose, saccharose, and raffinose, coagulates milk, and usually ferments salicin. Twenty-two members of this group were isolated. A point of some interest is as to how far streptococci from the same milk sample were identical. This is in part shown in Table 3. As a rule it may be said that the streptococci from any one sample were either identical or only differed in one characteristic, but exceptions to this were not infrequent. A typical example of this is cow 33. Six streptococci were isolated from this cow; of these five were identical, while the sixth only differed in that it failed to ferment salicin.

The pathogenicity of many of these streptococci was tested upon mice, most of them by subcutaneous, a few by intraperitoneal inoculation. Where a good growth in broth resulted, 1 cc. of a two days old broth culture was usually employed, but when the broth growth was scanty an emulsion of an agar growth in sterile water or agar condensation fluid was used. The streptococci tested

in this way are shown in Table 3 (last column). Where streptococci from the same milk were tested a mixture of the cultures was used for the one injection.

The virulence of 25 streptococci were in this way investigated. *In every case the results were negative.* In one instance the mouse died 15 days after intraperitoneal injection, but a coli group organism was recovered in pure culture. This was doubtless an accidental infection, and possibly due to injury to the gut in the injection.

*The relationship of the presence of streptococci to local conditions of the cow.*—If the mere presence of streptococci as a class is considered, no relationship is to be detected between their presence and the existence of local conditions, the number of calves or the stage of pregnancy.

There does seem, however, to be a connection between local conditions and the presence of a certain kind of streptococcus.

This streptococcus is a long, or very long, chain form which grows in broth as a clear, or almost clear, growth with a marked deposit. It stains by Gram's method and grows at both 21°C. and 37°C. It clots milk within three days. Lactose, saccharose, and raffinose are fermented and salicin usually, while mannite, coniferin and inulin remain unaffected. It grows upon gelatin and agar in the typical streptococcus manner and without liquefaction of the gelatin.

The milk samples in which this streptococcus were found are shown in the following table:—

Cow.	Udder and teat condition.	Number of streptococci upon the agar plates.	Characters of the streptococci as regards fermentation of		Remarks.
			Salicin.	Raffinose.	
17	Nil abnormal .. ..	Very abundant ..	+	+	One did not ferment salicin.
19	An ulcerated teat .. ..	Very abundant ..	+	+	
21	Nodules at base of two of the teats.	Scanty .. ..	+	+	
33	An ulcerated teat .. ..	Very abundant ..	+	+	One did not ferment salicin.
42	Nil abnormal .. ..	Very abundant ..	±	-	
43	Nil abnormal .. ..	In moderate numbers	+	-	
49	Extensive teat ulceration	In moderate numbers	-	+	

This table shows that streptococci of this kind were isolated from seven cows, and that in most cases they were present in large numbers. Allied organisms but fermenting one or another substance were found in several other cases.

In four instances some local condition was detected, three being ulcerated teats.

When the infrequency of the presence of local conditions in the cows examined is considered this is not without significance. Apart from the cases in the table, in only two other instances

were local conditions detected, *i.e.*, in cows 3 and 5. In the former a hard mass was present in one quarter, while in the latter one teat was tender, but without external ulceration. In neither of these cases was the milk deposit brushed over agar plates.

Also in cows No. 42 and No. 43, in which nothing abnormal could be detected, the streptococci isolated were not quite identical with the others. They produced chains of only moderate length and a more turbid growth in broth.

It is certainly of interest that in the only three cases associated with ulcerated teats, streptococci of the kind described were isolated from the milk and were present in marked abundance. This association may be accidental, but it is more probably a direct relationship of cause and effect. Which, however, is the primary condition is not evident.

It may be that the frequent passage of milk loaded with these streptococci causes cracks and abrasions of the teat to become infected and so sets up the ulceration, or, on the other hand, the teat condition may be primary, and the presence of these organisms upon the ulcerated surfaces of the teat may, by direct transmission, infect the teat ducts and milk cistern. The relationship of these streptococci to the streptococci found in cases of mastitis is more conveniently considered in the second part of this report.

2. *Staphylococci*.—As already shown in Table 2 these organisms were present in practically every milk examined.

The ordinary white and brown and to a certain extent the yellow staphylococcus must be regarded as very frequent, almost habitual, organisms in milk drawn direct from healthy cows. The pathogenicity of six of them was tested with uniformly negative result. A number were sub-cultivated and their characters fairly completely determined. The results are recorded in Table 5. This table shows that they also could be classified into a large number of different varieties. No relationship to any special local conditions could be made out.

I regard these organisms as naturally present in milk samples even when drawn direct from the udder. In general they are probably without significance, but they have this importance, that unless their normal presence is recognised, a causal significance may be incorrectly attached to their detection in slightly abnormal conditions.

Of greater interest is the presence of quite another variety of staphylococcus, the organism which is spoken of in Table 2 as a *translucent staphylococcus*.

Organisms of this group differ essentially from the ordinary staphylococci and form a group between these and streptococci. They were present in a great many of the samples. The characters of 30 such organisms, isolated from 14 different cows, are described in Table 4.

Certain well-defined characters differentiate them sharply. They all grow readily upon agar plates, producing minute colonies about the same size or slightly smaller than those of streptococci. These colonies are semi-translucent, but are distinctly less translucent than those of streptococci.

TABLE 4.

Number.	Source.	Morphology.	Characters of broth growth.	Acid in 3 days in							Coagulation of milk with gelatin at 50°C.
				Lactose.	Saccharose.	Mannite.	Salicin.	Conferin.	Raffinose.	Inulin.	
1	Cow 14	Cocci in quite small groups.	No growth ..	-	+	-	-	-	-	-	+
2	R. F. Q.	Cocci in quite small groups.	Clear: slight deposit.	-	-	-	-	-	-	-	+
3	Cow 22	Cocci in quite small groups.	No growth ..	+	-	-	-	+	-	-	-
4	Cow 22	Cocci in quite small groups.	No growth ..	+	+	+	-	-	-	-	-
5	Cow 22	Cocci in quite small groups.	Clear: very slight deposit.	+	+	+	+	+	-	-	-
6	Cow 23	Cocci in quite small groups.	No growth ..	+	-	-	-	+	-	-	+
7	Cow 23	Cocci in quite small groups.	No growth ..	+	+	-	-	-	-	-	+
8	Cow 23	Cocci in quite small groups.	Clear: very slight growth.	+	+	-	+	+	+	-	+
9	Cow 24	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
10	Cow 24	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
11	Cow 24	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	+	+
12	Cow 25	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
13	Cow 25	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
14	Cow 25	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	+	+
15	Cow 25	Cocci in quite small groups.	No growth ..	+	+	+	+	+	+	-	+
16	Cow 26	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
17	Cow 26	Cocci in quite small groups.	Clear: slight deposit.	+	+	-	+	+	+	+	+
18	Cow 31	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
19	Cow 31	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
20	Cow 31	Cocci in quite small groups.	Clear: slight deposit.	+	+	+	+	+	+	-	+
21	Cow 32	Cocci in quite small groups.	Clear: deposit ..	+	+	+	+	+	+	-	+
22	Cow 32	Cocci in quite small groups.	Clear: deposit ..	+	+	+	+	-	-	+	+
23	Cow 35	Cocci in quite small groups.	No growth ..	+	+	+	+	+	-	-	+
24	Cow 35	Cocci in quite small groups.	Clear: deposit ..	+	+	+	+	+	+	-	+
25	Cow 35	Cocci in quite small groups.	Clear: deposit ..	+	+	+	-	+	+	-	+
26	Cow 38	Cocci in quite small groups.	No growth ..	-	-	-	-	-	-	-	+
27	Cow 38	Cocci in quite small groups.	No growth ..	-	-	-	-	-	-	-	+
28	Cow 38	Cocci in quite small groups.	No growth ..	+	-	-	-	-	-	-	+
29	Cow 39	Cocci in quite small groups.	Clear: deposit ..	+	+	+	+	+	+	-	+
30	Cow 26A	Cocci in quite small groups.	Clear: deposit ..	-	-	-	-	-	-	-	+

Upon agar slope they produce a semi-translucent growth of discrete minute colonies similar to those upon the agar plate. In this they closely resemble the growth of streptococci, and are quite unlike the opaque, white or coloured growth of the ordinary staphylococci.

TABLE 5.

Number.	Source.	Morphology (in broth cultures).	Characters of broth growth.	Acid in 3 days in							Coagulation of milk.	Liquefaction of gelatin.	Colour of growth upon agar slope.
				Lactose.	Saccharose.	Mannite.	Salicin.	Coniferin.	Raffinose.	Inulin.			
31	Cow 3	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow brown.
32	Cow 2	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow white.
33	Cow 4	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
34	Cow 5	Diplococci and groups of cocci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Orange.
35	Cow 7	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow.
36	Cow 7	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow white.
37	Cow 7	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow.
38	Cow 7	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow.
39	Cow 14	Diplococci and cocci in groups	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow.
40	Cow 15	Diplococci and cocci in groups	...	Nearly clear : marked deposit	...	...	...	...	...	...	++	+	Yellow white.
41	Cow 15	Diplococci and cocci in groups	...	Nearly clear : marked deposit	...	...	...	...	...	...	++	+	Yellow white.
42	Cow 17	Cocci in groups	...	Nearly clear : marked deposit	...	...	...	...	...	...	++	+	White.
43	Cow 17	Cocci in groups	...	Clear : marked deposit	...	...	...	...	...	...	++	+	Yellow white.
44	Cow 19	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
45	Cow 21	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
46	Cow 23	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
47	Cow 23	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
48	Cow 27	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
49	Cow 27	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow.
50	Cow 26	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	Yellow.
51	Cow 28	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.
52	Cow 28	Diplococci	...	Uniform turbidity	...	...	...	...	...	...	++	+	White.

Upon gelatin slope they grow as minute white circular colonies and without liquefaction. In a few instances no growth upon gelatin (at 21° C.) took place.

Their growth in broth is also characteristic. They either do not grow at all, or if growth takes place it is scanty and shown only as a slight deposit, the broth above remaining perfectly clear.

Their morphology, examined either in broth (if any growth took place) or more usually in agar condensation fluid, is always the same—small cocci present in quite small groups, often not more than 8 to 12 cocci. In a few some diplococci were also present, but the quite small groups is the characteristic feature. Chains of cocci were never met with.

Milk is never coagulated, although usually a slight amount of acid is produced.

In all these characters they were practically identical, but by means of Gordon's sugar-alcohol tests they could be shown to comprise a large number of different varieties ranging from those which fermented all seven of the substances tested to those which fermented none of them.

The pathogenicity of several strains was tested upon mice, always with negative result.

I believe these translucent staphylococci are without significance. In reports it is not infrequently recorded that minute colonies on agar plates, which appeared to be those of streptococci on sub-cultivation into broth showed no growth. These results are, I believe, frequently due to the fact that the colonies sub-cultivated were these translucent staphylococci. If the sub-cultivation had been made upon sloped agar, growth would have resulted.

3. *Diphtheroid bacilli*.—From certain of the milk samples bacilli were met with which in their morphological characters, particularly when stained by watery methylene blue, closely resembled diphtheria bacilli. They were not specially looked for but were met with in the milk of six healthy cows and in three cases of mastitis.

The presumably healthy cows in which they were found were Nos. 13, 14, 15, 17, 21 and 27. It is of interest to note that cows 13, 14, 15, 17 were from the same cowshed, which contained only five cows in all.

The bacilli are straight or slightly curved, and frequently distinctly clubbed. Stained by methylene blue, they show well-marked beading and frequently a granule at one end; the other end is always more or less pointed. They show much variation in size. The darkly stained granules are rendered very distinct in watery methylene blue preparations, by running in dilute acetic acid under the coverslip, exactly in the same way as for *B. diphtheriæ*.

No blue granules are found when stained by Neisser's stain, but they retain the stain in Gram's method.

Non-motile in hanging drop preparations.

Upon agar slope they grow rapidly, forming very delicate minute translucent circular colonies. Similar colonies are produced upon agar plates.

Upon blood serum they grow as white or yellow-white minute raised circular colonies. The microscopic preparations made from blood serum growths were less like *B. diphtheriæ* than those prepared from growths on agar media.

In broth no change is seen for two to three days, but after three to five days' incubation a delicate flocculent precipitate can be made out on shaking the tube. The supernatant broth remains quite clear. In some instances no growth in broth could be obtained.

Little or no growth takes place upon gelatin slope media. They grow in litmus milk, but without visible change.

Some eight organisms were tested as regards their action upon the sugar-alcohol media. Several produced a little acid in the lactose tubes, but the other substances were never fermented.

Injected into mice they were non-pathogenic.

An account of this organism, or group of organisms, is introduced here because of their morphological resemblance to *B. diphtheriæ*, but they are apparently devoid of significance, and were not found to be associated with any local condition or peculiarity of the cows in which they were found.

*C. The source of streptococci and staphylococci in the individual milk samples.*—It might possibly be urged, since special precautions to ensure absolutely aseptic conditions were not taken, that these organisms obtained access to the milk after it left the cow, and were not derived from the udder or teat canals.

While a few organisms may have gained access in this way, this source, for the bacteria found, is certainly exceptional. The milk was milked directly into the bottle, which had a quite narrow neck. The plates made from the centrifuged deposits frequently showed abundant colonies, in numbers quite too numerous to be explained by accidental outside contamination.

The kinds of organisms present were not those which would be likely to be found if the contamination was from outside; thus the streptococci were mostly of the *longus* type (unlike the streptococci of cow dung) and *B. coli* was only very rarely present.

The vast majority of the streptococci and staphylococci were undoubtedly present in the milk while in the teats and milk cistern.

The samples were all of middle milk, and from the fore milk organisms are still more numerous. These investigations, therefore, show that there is extensive infection of the milk before it leaves the udder, and not a mere lodgment of a few organisms in the teat canals.

The results of Ward and of Uhlmann show the same thing in another way. Ward\* examined the udders of 19 freshly slaughtered milch cows, and found that the lactiferous ducts of all 19 harboured bacteria throughout their whole extent.

Uhlmann† cut sections of the teats of 35 cows. In all the sections he found bacteria, mostly only a few, occasionally hundreds.

The source of these bacteria is probably to be explained as due to direct infection through the open teat mouth, spread and transmitted by the traces of milk in the canal. Some observers (D'heil, etc.) maintain that a column of milk stands in

\* A. R. Ward, 1900. Bulletin, No. 178. Cornell University Agricultural Experimental Station.

† O. Uhlmann, 1903. Centralblatt f. Bakt. 1, XXXV.

the teat ducts, but apart from this the possibilities of infection are obvious.

Their significance has been dealt with. The almost invariable presence of staphylococci and of many streptococci shows that these must be, for the most part, harmless to man, while Table 6 shows that, for all the tests made, they are harmless to mice.

It is, however, quite possible that certain kinds, such as the long chain form associated with sore teats, may possess some pathogenic properties, a possibility worthy of further inquiry.

TABLE 6.—MICE FED or INOCULATED with MILK from INDIVIDUAL COWS.

Cow.	Nature of Experiment.	Dose.	Result.
No. 13	Feeding ... ..	In every case the same, i.e., as much as possible of the deposit of 10 cc. of the milk centrifuged for 10 minutes.	No effect.
No. 14	Feeding ... ..		No effect.
No. 15	Feeding ... ..		No effect.
No. 16	Feeding ... ..		No effect.
No. 17	Feeding ... ..		No effect.
No. 19	Feeding ... ..		No effect.
No. 20	Feeding ... ..		Died 7 days later. MG, recovered in pure culture.*
No. 24	Feeding ... ..		No effect.
No. 21	Subcutaneous injection ...		No effect.
No. 22	Subcutaneous injection ...		No effect.
No. 23	Subcutaneous injection ...		No effect.
No. 25	Subcutaneous injection ...		No effect.
No. 27	Subcutaneous injection ...		No effect.
No. 28	Subcutaneous injection ...		No effect.
No. 29	Subcutaneous injection ...		No effect.
No. 46	Intraperitoneal injection..		No effect.

\* This was an accidental infection, and is dealt with in the Report upon the Distribution of Organisms of the Gaertner Group. See page 253 of this Report. MG, a glucose fermenting bacillus of the Gaertner group, was not present in the inoculated milk.

### SECTION III.

#### *B. coli* and other glucose fermenting organisms in milk.

Table 7 shows how far these organisms were present in the samples. Out of 52 samples from mixed quarter milks these organisms were found only in four instances. In one of these, cow No. 49, the examination of the separate quarters, a few days previously, had shown the presence of *B. coli* in three out of the four samples. In this case it is probable that they were in the milk before it left the teats, but for the others it is more likely that they obtained access from outside, due to accidental infection, strict aseptic precautions not being taken.

In any case these results show that *B. coli* is not found, except very rarely, in cleanly collected milk samples, and that, therefore, its presence may be taken as an indicator of outside contamination.

The characters of the organisms found are given in Table 8. They show nothing of special interest. All are ordinary typical *B. coli* except No. 2 which is a *B. lactis aerogenes*.

TABLE 7.—PRESENCE of GLUCOSE FERMENTERS in the MILK SAMPLES.

Cow.	Glucose fermenters (acid and gas) in		Cow.	Glucose fermenters (acid and gas) in	
	1 cc.	10 cc.		1 cc.	10 cc.
1	+	+	29	—	—
2	—	—	30	—	—
3	—	—	31	—	+
4	—	—	32	—	—
5	—	—	33	—	—
6	—	—	34	—	—
7	—	—	35	—	—
8	—	—	36	—	—
9	—	—	37	—	—
10	—	—	38	—	—
11	—	—	39	—	—
12	—	—	40	—	—
13	—	—	41	—	—
14	—	—	42	—	+
15	—	—	43	—	—
16	—	—	44	—	—
17	—	—	45	—	—
18	—	—	46	—	—
19	—	—	47	—	—
20	—	—	48	—	—
21	—	—	49	—	+
22	—	—	50	—	—
23	—	—	33A	—	—
24	—	—	26A	—	—
25	—	—	49 R. H. Q.	—	+
26	—	—	49 L. H. Q.	—	+
27	—	—	49 R. F. Q.	—	+
28	—	—	49 L. F. Q.	—	—

TABLE 8.—CHARACTERS of GLUCOSE FERMENTERS ISOLATED.

Number.	Source.	Fermentation of				Litmus milk.		Neutral red reaction.	Liquefaction of gelatin.	Indol.	Gelatin slope.
		Glucose.	Lactose.	Dulcitate.	Saccharose.	Acid.	Coagulation.				
1	Cow No. 1	+	+	+	+	+	+	+	—	+	Bluish translucent growth.
2	Cow No. 31	+	+	+	+	+	+	+	—	—	White opaque growth.
3	Cow No. 42	+	+	+	—	+	+	—	—	+	Bluish translucent growth.
4	Cow No. 49	+	+	—	+	+	+	—	—	+	Bluish translucent growth.
5	Cow No. 49 R. H. Q.	+	+	—	+	+	+	+	—	+	Bluish translucent growth.
6	Cow No. 49 L. H. Q.	+	+	—	+	+	+	—	—	+	Bluish translucent growth.
7	Cow No. 49 R. F. Q.	+	+	—	+	+	+	—	—	+	Bluish translucent growth.

## SECTION IV.

*Leucocytes in the milk of individual cows.*

It is generally accepted that normal milk may contain some leucocytes. On the other hand it is frequently stated that if milk contains pus cells it is unfit for human consumption. These two statements imply that there is a definite difference between a pus cell and a leucocyte, so that the one can be detected and the milk excluded, while the other can be recognised as a natural constituent of milk. Such a distinction cannot be maintained. In pus the cells of the purulent exudate are in the main polymorphonuclear in character, but in quite healthy milk many of the leucocytes present are also polymorphonuclear. It is true that the pus cell gradually becomes disintegrated, with fat formation, the nuclei sharing in the changes, so that its characters become less distinctive, but these changes frequently are not present and are in no way sufficient to differentiate between an ordinary leucocyte and a pus cell.

Failing distinction in character the determination of the presence of pus in milk must rest upon the numerical presence of leucocytes in that fluid. This at once raises the question as to what number of leucocytes in milk may be considered sufficient to allow a diagnosis of pus in milk to be made.

In extreme cases there is no difficulty. In many cases of mastitis the milk drawn from the affected quarters is obviously purulent to the naked eye and microscopically shows leucocytes in extreme abundance. On the other hand, some samples of healthy milk show scarcely any leucocytes even in microscopic preparations made from centrifugalised deposits. One milk is obviously purulent, the other is as obviously free from pus. Between these two conditions many gradations are met with.

In addition to enumerations of the number of leucocytes present an endeavour was made to differentiate the different kinds and to make an enumeration of these, on the lines of the differential enumeration of the white corpuscles of blood.

Such a differentiation was found to be easy in some cases, but a matter of difficulty in others, and the results obtained can only be accepted as very approximate data. A considerable number of different kinds of leucocytes were found, but the great majority could be broadly classified into one of three groups. A broad classification of this kind was found to be more practicable than a more careful sub-division and enumeration of many sub-groups.

The enumerations were all made from centrifugalised milk deposits, stained with Löffler's methylene blue, and examined with  $\frac{1}{17}$  oil immersion lens, after mounting in balsam.

The leucocytes present in ordinary milk samples may be classified into the three following groups:—

- (a) *Polymorphonuclear leucocytes*.—Medium sized leucocytes with a diameter varying from about 7.5–10 $\mu$ . The protoplasm of the cell is only seen with difficulty, but

the nucleus stains deeply, and from its twisted shape appears frequently like several nuclei.

- (b) *Lymphocytes*.—Considerably smaller. They show great variability in size, in some cases being quite small, but for the most part they have a diameter of about 5–7 $\mu$ . They are circular, or nearly so; the nucleus stains deeply and occupies nearly the whole of the cell.

- (c) *Large leucocytes*.—These cells also vary in size, but on the whole are considerably larger than any of the other varieties of leucocytes met with in milk. Their average diameter is from 13–16 $\mu$ , but they may be as large as 24 $\mu$  or more. They have a single nucleus, usually staining well, which occupies only a relatively small proportion of the cell, the rest being lighter staining vacuolated protoplasm. While generally well defined and distinct, occasionally all gradations between these cells and lymphocytes are met with.

*A. The general presence of leucocytes in milk.*—The number of leucocytes in the 52 milk samples examined with a differential count of the different kinds present is shown in Table 9, while in Table 1, the number of leucocytes in relation to the physiological and pathological condition of the cow is considered. As these tables show, leucocytes were present in every sample of milk examined. The numbers present showed very great variety, the actual figures obtained varying from 14 to 4,100 per cubic millimetre.

To arrive at the significance of leucocytes in milk it is important to consider if they are associated with definite physiological conditions, such as recent calving, pregnancy, &c.; or with pathological conditions, such as udder injuries, inflamed teats, old or present udder inflammation. Some of the more important physiological conditions will first be considered.

(1) *The number of calves and age of the cow.*—The number of calves could generally be ascertained, while the exact age of the cow was not usually attainable. The number of calves, however, furnishes a good guide as to the approximate age of the cow. Table 1 shows that although many of the cows with only one calf yielded very low counts, 50, 150, 28, &c., leucocytes per cubic millimetre, yet in several instances rather high counts were met with.

Excluding cows with local pathological conditions, or which were drying off, &c., the average number of leucocytes—

In the cows with one calf was 190 (50, 540, 150, 230, 28, 130, 56, 670, 84, 64, 85).

In the cows with two calves was 217 (520, 85, 250, 14).

In the cows with four or more calves was 168 (270, 180, 50, 85, 43, 330, 105, 200, 100, 320).

The age of the cow and the number of calves has evidently little or no influence upon the number of leucocytes.

TABLE 9.

Number of the cow.	Number of leucocytes per cubic mm.	Varieties of leucocytes (percentages).				Local conditions present.
		Polymorpho-nuclear.	Lymphocytes.	Large leucocytes.	Other kinds.	
1	285	15	25	55	5	Very little milk given. A few pints only of milk.
2	3,610	6	24	70		
3	1,230	10	5	85		
4	50	—	—	—	—	
5	3,320	75	5	20	—	Tender test.
6	210	5	90	5		
7	80	76	12	12		
8	270	70	15	15		
9	106	40	45	15		
10	180	70	20	10		
11	540	6	90	4		
12	150	78	5	17		
13	230	83	10	7		Old udder injury.
14	2,010	65	0	33	2	
15	50	30	45	25		
16	85	—	—	—		
17	330	86	6	5	3	Milk diminishing ; an ulcerated test. Nodules in udder.
18	520	68	10	22		
19	250	70	0	30		
20	2,650	87	1	12		
21	3,700	55	0	45		History of old inflammation.
22	28	10	50	40		
23	50	16	80	4		
24	85	5	85	10		
25	1,600	50	20	30		Amount of milk diminishing. About $\frac{1}{2}$ gallon a day only.
26	270	22	58	20		
27	430	70	5	25		
28	560	75	5	20		
29	940	13	12	75		One test ulcerated.
30	130	40	50	10		
31	56	—	—	—		
32	200	70	15	15		
33	105	—	—	—		Amount of milk diminishing. Amount of milk diminishing. Only 2-3 pints yielded per day.
34	670	90	2	8		
35	84	35	47	12	6	
36	660	20	60	20		
37	490	45	30	20	5	
38	4,100	25	5	10	60	
39	200	82	1	17		
40	250	70	25	5		
41	460	55	5	40		" Milk fever " 5 months previously.
42	85	98	3	4		
43	295	50	17	30	3	
44	100	—	—	—		
45	64	40	30	25	5	Calved 32 hours previously. Extensive test ulceration.
46	1,025	60	5	35		
47	270	25	65	10		
48	1,205	8	72	20		
49	14	12	85	3		Calved 3 days previously.
50	85	—	—	—		
53A	132	—	—	—		
26A	320	20	60	20		

(2) *The part of the milk stream.*—The results of some experiments previously carried out showed that the number of leucocytes in the middle milk is always less than the number in either the fore milk or strippings.

All the samples recorded from individual healthy cows were middle milk samples.

(3) *The period since calving.*—No relationship between the period since calving and the number of leucocytes is apparent, except for animals which have only calved within a day or so. Thus for cow No. 48, calved 32 hours previously, the number of leucocytes was 1,205, but for all cows which had calved a week or more, no influence on the number of leucocytes could be made out.

(4) *The stage of pregnancy.*—The earlier stages of pregnancy do not seemingly influence the number of leucocytes, but in the later stages when the milk is diminishing a rise in the number of leucocytes almost invariably takes place.

In the following table the cows due to calve within 2-3 months are grouped together:—

Cow.	Time to next parturition.	Amount of milk yielded.	Number of leucocytes.	Varieties of leucocytes (percentages).			
				Polymorpho-nuclear.	Lymphocytes.	Large leucocytes.	Other kinds.
No. 2	2 months	Almost nil ... ..	3,610	6	24	70	
No. 3	3½ months	A few pints ... ..	1,230	10	5	85	
No. 19	2 months	Diminishing. Milked only once a day.	250	70	0	30	
No. 28	3 months	Diminishing ... ..	560	75	5	20	
No. 29	2 months	About ¼ gallon a day	940	13	12	75	
No. 31	2 months	Abundant ... ..	56	—	—	—	
No. 36	7 weeks	6-8 pints only a day	660	20	60	20	
No. 37	8 weeks	8-10 pints only a day	490	45	30	20	5
No. 38	2 months	2-3 pints only a day	4,100	25	5	10	60

This table shows that the diminution of milk is associated with a rise in the number of leucocytes, and the less the milk yielded the higher the number of leucocytes.

In cow No. 31, the milk was said to be abundant, and probably a mistake was made in computing the time to calving.

The milk of cows "drying off" is, therefore, rich in leucocytes.

The increase in the number of leucocytes is especially shown in an increase in the percentage of "large leucocytes." In three out of the four counts over 800, they formed over 70 per cent.

(5) *Association with pathological conditions.*—In cases of mastitis the number of leucocytes in the milk is enormously increased (see Table 13, page 243 of this report). Even in cases in which the quarter does not undergo necrosis but apparently completely recovers, this condition does not subside rapidly, and months after the inflammation, when the milk to the naked eye is quite normal, the number of leucocytes may be found to be high.

Thus in a case of mastitis (antecedent to the cases reported in this Report) the fluid from the affected quarter, two months after the attack, showed 12,200 leucocytes per cubic mm., although the milk looked normal. The milk from the same cow, but in this case from all four quarters, examined seven months after the attack, showed 1,190 leucocytes.

Antecedent mastitis might, therefore, be expected to be a cause of high leucocyte count.

In all the cows investigated a careful examination was made of the udders and teats, while inquiry was made as to previous inflammation or injuries. The results of these examinations and inquiries are recorded in Table 1 with other details.

A consideration of this table shows that, apart from a high count due to cows drying off, there is a close relationship between the presence of numerous leucocytes, and either some present pathological condition or a definite history of some antecedent one.

To bring this out all the cows with a count over 800 may be briefly recapitulated.

The high counts of cows 2, 3, 29, 38 are explained by the diminution of the milk and the "drying off" of the cows.

The other cases are:—

- |         |                   |   |
|---------|-------------------|---|
| No. 5.  | 3,320 leucocytes. | A tender teat evidently due to some local inflammation.   |
| No. 14. | 2,010 leucocytes. | History of udder injury by another cow some three months previously; the quarter affected yielding a diminished quantity of milk.   |
| No. 20. | 2,650 leucocytes. | Nil to be found abnormal, and no history of previous injury or inflammation. The teats were very large.   |
| No. 21. | 3,700 leucocytes. | Two nodules each about the size of a small walnut at the base of the left fore and left hind teats. Not tender. No previous injury or inflammation admitted.  |
| No. 25. | 1,600 leucocytes. | Nil on examination. History of old inflammation of the left hind quarter about a year previously. The milk was affected, but the resultant swelling was said to have subsequently quickly subsided.   |
| No. 46. | 1,025 leucocytes. | Nil abnormal to be detected on examination. A very definite history of "milk fever" at the time of last calving in October, 1906, was obtained. At that time the right hind quarter was affected, being painful and yielding no milk. The other quarters were said to have been unaffected. |

The only other cow with a count over 800 is No. 48, with a count of 1,205. This cow had only calved 32 hours previously, and the milk was not being added to that of the herd, while the teats were hot and slightly tender.

It will be seen that, with the exception of cow No. 20, the high number of leucocytes was definitely associated with either present or past local mischief. The histories were received and the examinations made at the time of collection of the samples and before, therefore, the number of leucocytes was estimated.

In seven instances a separate examination of the milk from each quarter was made, five out of the six cases just enumerated being included. The results are shown in Table 10:—

TABLE 10.

Number of the cow.	Individual quarters examined.	Number of leucocytes per cubic mm.	Varieties of leucocytes (percentages).				Local conditions present.
			Polymorpho-nuclear.	Lymphocytes.	Large leucocytes.	Other kinds.	
14	Right Fore ..	170	70	24	6		This quarter injured 3 months previously. Now gives diminished milk.
"	Right Hind ..	2,310	10	0	90		
"	Left Fore ..	85	65	10	25		
"	Left Hind ..	330	53	4	40	3	
17	Right Fore ..	180	35	25	40		
"	Right Hind ..	1,560	90	3	7		
"	Left Fore ..	180	50	18	30	4	
"	Left Hind ..	330	75	5	20		
20	Right Fore ..	2,210	70	25	5		
"	Right Hind ..	780	87	5	28		
"	Left Fore ..	1,140	70	5	20	5	
"	Left Hind ..	320	45	25	25	5	
21	Right Fore ..	120	60	30	10		
"	Right Hind ..	520	75	5	20		
"	Left Fore ..	140	60	25	10	5	
"	Left Hind ..	100	55	30	15		
25	Right Fore ..	90	57	25	18		Nodule size of a walnut at base of each of these teats.
"	Right Hind ..	350	60	30	10		
"	Left Fore ..	1,330	93	2	5		
"	Left Hind ..	3,820	48	25	27		
48	Right Fore ..	280	10	65	25		Old inflammation in this quarter only, about a year previously.
"	Right Hind ..	2,250	40	0	60		
							This quarter only, painful and inflamed at the time of the milk fever, and for a few days giving no milk.

TABLE 10—*continued*.

Number of the cow.	Individual quarters examined.	Number of leucocytes per cubic mm.	Varieties of leucocytes (percentages).				Local conditions present.
			Polymorpho-nuclear.	Lymphocytes.	Large leucocytes.	Other kinds.	
48	Left Fore ..	28	20	40	40		} Extensive teat ulceration: teats not tender.
"	Left Hind ..	64	15	50	35		
49	Right Fore ..	14	0	0	100		
"	Right Hind ..	35	0	95	5		
"	Left Fore ..	14	0	90	10		
"	Left Hind ..	21	0	100	0		

The results obtained, while not giving quite uniform results, are very suggestive.

Thus for cow No. 14, the only quarter with a high count was the right hind quarter with a count of 2,310, the quarter giving a definite history of previous inflammation. The injury to this quarter is evidently the sole cause of the large number of leucocytes yielded by the mixed quarters sample.

So for cow No. 25, the quarter with the highest count is the left hind quarter, the one for which a definite history of old inflammation was received. Also for cow No. 46, the history obtained showed that the right hind quarter had been affected, the quarter yielding a count of 2,250 leucocytes per cubic mm.

Cow No. 49 is of no special interest, except that it shows that the extensive teat ulceration had not affected the glandular part of the udder. No. 17 was also from a cow which did not show a high count for the mixed quarters milk. The milk from one quarter showed 1,550 leucocytes per cubic mm. Nothing was known to account for this.

In regard to the other two animals (Nos. 20 and 21), they were both from cows examined in the course of a general inspection, and were obtained from a cowkeeper who would not admit that anything had ever been the matter with any of his cows. Except No. 49, the milk of the other cows was obtained from two cowkeepers who were specially interested in the inquiry and who readily afforded every assistance.

It is not unreasonable to suppose that, if the other two cows had been from the same sheds and a full history had been obtainable, a satisfactory explanation of the conditions found would have been forthcoming.

In regard to cow No. 21, I am unable to explain the high mixed quarters count and the low separate counts. The latter were

collected seven days subsequently, and it might possibly be that some local inflammatory condition was present which subsided before the second collection. Nothing of the kind was, however, detected, and the quarters with the nodules at the base of their teats both showed low counts.

In view of the difficulties experienced in obtaining histories of old injuries, inflammation, &c., these results are very striking, and show clearly the close relationship between local pathological conditions and the high leucocyte count.

*B. The different kinds of leucocytes in relation to local conditions.*—A differential classification of the leucocytes in the stained deposits of the different milk samples was made in every case, except those in which the leucocytes were too few to allow this to be made. The results are shown in Table 9.

Table 13 (page 243), dealing with the results of the examination of cows suffering from garget, shows that the proportion of the different kinds of leucocytes present in the actually affected quarters approximated to those found in pus. In ordinary pus the polymorphonuclear leucocytes are the most characteristic and abundant feature. Thus in a determination of typical abscess pus 88 per cent. of the cells were of this variety, and about 5 per cent. lymphocytes.

Table 9 shows that this relative proportion is also, in the main, shown for the cows with local conditions, pointing to present or past inflammation. Thus for the cows with high counts associated with such conditions, Nos. 5, 14, 20, 21, 25 and 46, the percentage of polymorphonuclear leucocytes was respectively 75, 65, 87, 55, 50, 60.

These results are therefore quite different from those of the cows with high counts associated with diminishing milk due to approaching parturition.

Unfortunately a similar relative proportion of the different leucocytes to that found in many suppurative conditions is met with in many milk samples from perfectly healthy cows, cf. Nos. 7, 8, 12 and many others.

It cannot therefore be said either that the presence of any one particular kind of leucocyte, or its presence in any special proportion, is characteristic and diagnostic of pus in milk.

I am not prepared from the data available to set up an arbitrary standard of the number of leucocytes necessary to warrant a diagnosis of the presence of pus, but certainly more than 800 per cubic mm. warrants a careful inquiry into the existence of local conditions.

*C. The relationship of the number of leucocytes to the presence of streptococci.*—Bergey traced a relationship between the number of pus cells and the number of streptococci in milk. The results here recorded show no such relationship.

If the mere finding of streptococci, as shown by their presence in broth to which milk has been added, be considered, the presence of streptococci was not in any way associated with a large

number of leucocytes in the milk. This is brought out in the following Table :—

Number of leucocytes per cubic mm.	Number of samples.	Streptococci present in 1 cc.	Streptococci absent in 1 cc.	Percentages of streptococci.	
				Present in 1 cc.	Absent in 1 cc.
Less than 300 ...	28	18	10	64	36
300-1,000 ...	7	4	3	57	43
More than 1,000 ...	9	7	2	78	22

Also the presence of streptococci in abundance had no relationship to the number of leucocytes present. In only seven cases were streptococci colonies very abundantly met with, *i.e.*, cows Nos. 17, 19, 20, 29, 33, 42, 33a. The number of leucocytes present per cubic mm. for these seven cases was respectively 330, 250, 2,650, 940, 105, 85, 132. All gradations were met with.

It would seem that the presence of an excess of leucocytes and the presence of streptococci may be due to fundamentally different causes. The presence of an excess of leucocytes is seemingly definitely related to some present physiological or pathological condition, or to some antecedent pathological condition.

An excess of leucocytes may be due to the conditions associated with a diminution of the milk in the later stages of pregnancy. It may be due to some old injury or inflammation, all traces of which have been removed when ordinary methods of examination are used. Lastly, such an excess may be associated with some present pathological condition. Only in the last instance is an association with infective micro-organisms to be anticipated.

On the other hand the presence of streptococci is frequently a purely saprophytic phenomenon; in other cases it is probably associated with some local teat condition which does not affect the number of leucocytes, while only in certain cases is their presence in direct relationship to pathological conditions, causing a great increase in the number of leucocytes.

## PART II.

### A PRELIMINARY COMMUNICATION UPON THE BACTERIOLOGY OF "GARGET" IN COWS.

The condition called Garget or Mastitis is one which is by no means rare in ordinary milking cows.

As already mentioned the evidence connecting outbreaks of sore throat and possibly other conditions, with milk derived from an inflamed udder is, upon epidemiological grounds, very strong.

From a purely bacteriological standpoint, however, there appears to be little or no evidence, apart from tuberculosis, which has shown that the actual organisms which have set up mastitis in cows have also set up disease in human beings through the agency of milk.

Further general considerations render it highly probable that not all cases of udder inflammation have the property of rendering milk potentially infectious to those consuming it, even if it be granted that some have this property.

Outbreaks of disease spread by milk infected by cows acutely diseased are rare, and although this rarity is doubtless to some extent to be explained by the difficulty of tracing out such outbreaks and their liability to be altogether overlooked, yet, allowing for these, there is a discrepancy between the frequency of udder inflammations and the comparative infrequency of outbreaks traced to such conditions.

In other words, it is probable that only a certain proportion of cases of mastitis are really liable to serve as sources of human infection.

This is the more probable since the researches of different observers, especially of the Zurich and Bern schools of veterinary medicine have demonstrated that mastitis in cows may be set up by a number of different bacteria.

Also veterinary authorities, although widely differing as to their classifications of mastitis, yet all recognize different varieties and degrees.

This question is of much practical importance alike to those intrusted with the health of the community and to those financially interested in the dairy and allied industries.

Considered purely in its relationship to human pathology it is obviously of importance to ascertain :—

- (a.) If *all* cases of mastitis in cows are potentially capable of originating disease in human beings and if not to definitely discriminate between those which possess and those which do not possess this power.
- (b.) To consider the source of the bacteria which set up mastitis, and how far the condition can be prevented.
- (c.) In view of the fact that often the milk from the affected quarter only is rejected, to determine how far the causal agent is present in the milk of the apparently non-affected quarters.

The present report is only a preliminary communication and a more detailed consideration of the bacteriological findings and their relationship to human disease will be deferred to a subsequent report.

## SECTION I.

### *Methods Adopted.*

These were identical with those employed in Part I., only differing in minor matters sufficiently indicated in the text.

Although as far as possible "middle milk" samples were obtained from the different quarters it was not possible in every case to do this, since for the actually affected quarter usually only a very little fluid could be obtained, and it was all collected.

I am particularly indebted to Mr. W. F. Shaw, the Veterinary Inspector of the London County Council for supplying me with the fluid from Cases IV., V., VI., and for valuable notes upon the conditions of the cows.

## SECTION II.

The bacteriological and cytological results of the six cases examined.

*Case I.*—A Dutch cow ; has had three calves. Onset about five months after her last calf. The inflammation started in the right hind quarter. When seen by me four days after the onset of the inflammation the right hind quarter was hard and tense and very greatly enlarged. The other quarters were apparently not enlarged and, according to the veterinary surgeon who examined the case with me, were not affected.

The temperature of the cow was normal, and there was no evidence of disturbance of her general health.

The fluid from the affected quarter was fairly alkaline, and consisted of a slightly turbid brown whey containing abundant white stringy clots. It was scanty in amount and obtained with difficulty.

The milk from the other quarters was readily obtained and normal in appearance, except that from the left hind quarter which was slightly yellow.

This cow was re-examined 40 days later. The affected quarter was still markedly enlarged with indurated nodules in it, and yielded a turbid brown fluid containing clots. The fluid was rather more abundant than when first examined.

The other quarters were apparently healthy, and yielded milk which to the naked eye was quite normal.

The milk from these three quarters were being added to the milk obtained from the other cows in the sheds and sold in the ordinary way.

The mastitis had not extended to the other quarters.

*The cytological conditions present.*—The number of leucocytes found in the different quarters as well as the different kinds is shown in Table 13. This table shows that although the other quarters were apparently unaffected yet the milk yielded contained a large amount of pus. The re-examination 40 days later showed that this condition had in the main subsided in these quarters although the disease was still marked in the originally affected quarter. It may be mentioned, since it is not recorded in the table, that an estimation of the number of leucocytes in the left hind quarter was also made five days after the first samples were taken. The number per cubic mm. in this quarter was then 11,800.

The percentage of the varieties present were as far as possible made out, and the results show that in the cases with active suppuration the great majority of the leucocytes were of the polymorphonuclear variety corresponding to the condition found in ordinary human acute suppurations.

In the affected quarter at the first examination the leucocytes were considerably broken down, and a satisfactory differential count was impossible.

*The bacteriological results.*—The stained centrifugalised deposit of the fluid from the affected quarter (right hind) showed numerous chains of streptococci, the majority of which were short,

quite long streptococcus chains being present in only comparatively small numbers. Diplococci were also numerous. The centrifugalised deposits of the left hind quarter and the two front quarters showed numerous diplococci, short cocci chains, and long cocci chains. The long chains were decidedly more numerous than in the deposit from the affected quarter.

The deposits when re-examined 40 days later were quite different, those from the two hind quarters each showing an occasional diplococcus but no chains of cocci, while in those from the front quarters no organisms could be detected. 0.1 cc. of the affected quarter fluid added to broth and incubated at 37° C. showed next day abundant very long twisted chains.

Broth tubes inoculated with 0.1 cc. of the other quarter milks showed, when treated in the same way, abundant short and medium length chains.

To isolate the organisms present one loopful, and also 0.1 cc. of the fluid, from each of the three samples were spread over agar plates, and the resulting colonies examined after 24 and also after 40-48 hours incubation at 37° C.

All the sub-cultivations for the first examination were made into broth culture media.

*Organisms isolated from the affected quarter.*—Only a few staphylococcus colonies were present. The numerous minute colonies which developed were of two kinds. The one on sub-cultivation showed themselves to be streptococci, the other were organisms which would not grow in broth. These colonies were not further investigated from this sample, but a fresh sample was obtained five days later, and these minute colonies sub-cultivated directly upon sloped nutrient agar upon which they grew freely.

From the agar growth their morphological and cultural characters were investigated, and they were found to be bacilli morphologically closely resembling diphtheria bacilli, and quite similar to the organisms described in Part I. of this report as diphtheroid bacilli.

The characters of the streptococci isolated both from this and from the other quarters are set out in Table 11.

This table shows that streptococci were isolated from all the quarters, but that those obtained from the quarter mainly infected were sharply differentiated from those derived from the other quarters, by their inability to coagulate milk (within a week at 37° C.).

This table also shows that the streptococci isolated from the affected quarter, although not quite identical as regards their action upon the selected sugars and alcohols, yet were very nearly alike, a streptococcus forming medium and moderately long chains in broth, not coagulating milk and fermenting all the sugar-alcohols used, except inulin, being seemingly the common type.

The results of the examination 40 days later are of interest. The agar plates from 0.1 cc. of the fluid showed many staphylococcus and many streptococcus colonies. Eight of the latter on being sub-cultivated showed the character given in Table 11. Seven were quite identical, and all were quite different from the streptococci found at the onset of the attack.

Before considering the cause of this case of mastitis the inoculation results may be considered.

Three mice were each fed with bread soaked in the fluids from the affected quarter, the left hind quarter, and the mixed two front quarters.

The mouse inoculated with the milk from the two front quarters was ill next day and died 36 hours after the feeding, the other two mice were found dead the morning after feeding.

A careful post-mortem examination was made in each case, but no macroscopic lesions were found, and cultivations from the heart blood, peritoneal fluid, and spleen, upon agar slope, and into broth media remained sterile.

A mouse injected under the skin with 0.5 cc. of the fluid from the affected quarter died after six days. No streptococci were isolated, post-mortem, but the spleen was enlarged and congested, and an organism of the coli group fermenting glucose, but not lactose, and coagulating milk was isolated in pure culture. This was probably an accidental infection.

Inoculations were also performed with the samples obtained in the re-examination 40 days later.

A mouse fed with the centrifugalised deposit of 10 cc. of the milk from the left hind quarter showed no ill effects, while a mouse injected subcutaneously with the similar deposit from the left front quarter also remained unaffected.

Also a mouse fed with bread soaked with 1 cc. of the fluid from the affected quarter remained unaffected.

On the other hand a mouse injected under the skin with 0.5 cc. of this fluid died in 4 days. Nothing abnormal post-mortem, and the cultivations upon agar and into broth from the spleen and heart blood, remained sterile.

The same peculiarity of inability to isolate organisms from the dead mice was also shown by some of the inoculations with pure cultures.

*Streptococcus* No. 72 was injected subcutaneously into a mouse, the animal receiving about one quarter of an agar growth 24 hours' old. The animal died after five days, but nothing could be recovered, post-mortem and no macroscopic lesions were present.

In the same way a mouse fed with bread soaked in a broth culture of *Streptococcus* 74, died six days later, but no organisms could be recovered from the spleen, heart blood, or peritoneal fluid.

A mouse fed with a broth culture of *Streptococcus* 81, died after 30 hours, but no organisms could be recovered post-mortem.

On the other hand a mouse fed with *Streptococcus* 83, remained unaffected, while another mouse injected with *Streptococcus* 88, also remained unaffected.

These inoculation results are difficult to understand, and were quite unlike anything met with on other occasions.

The etiology of this case of mastitis is by no means clear. When examined within a few days of the onset, streptococci of the type of No. 72, &c. were certainly very abundant in the fluid from the affected quarter, and it is a reasonable supposition that they were the bacterial cause of the inflammation. The only other organisms

at all abundant were diphtheroid bacilli, bacilli quite identical with similar organisms obtained from quite healthy cows.

It is possible, but unlikely, that they played a part in the disease, and that it was a case of mixed infection. The streptococcus found at the last examination was a quite different organism, probably either saprophytic or a secondary infection.

This case illustrates the great difficulty sometimes experienced in arriving at the etiological cause of these cases.

TABLE 11.

Number.	Source.	Morphology (length of chains).	Characters of the growth in broth.	Acid in 3 days in							of coagulation milk.
				Lactose.	Saccharose.	Mannite.	Saltin.	Conferin.	Raffinose.	Inulin.	
72	Case I. R. H. Q.	Medium and long	Clear: deposit ..	+	+	+	+	+	+	-	-
73	"	Medium and long	Clear: deposit ..	+	+	+	+	+	+	-	-
74	"	Long .. ..	Clear: deposit ..	+	+	+	-	+	-	-	-
75	"	Long .. ..	Clear: deposit ..	+	+	+	+	+	-	-	-
76	"	Short and medium	Nearly clear: de- posit.	+	+	+	+	+	+	-	-
77	"	Short and medium	Slight turbidity ..	+	-	+	-	+	+	-	-
78	"	Long .. ..	Clear: deposit ..	+	+	-	+	+	+	-	+
79	Case I. L. H. Q.	Medium and long	Clear: deposit ..	+	+	-	-	+	+	-	+
80	"	Long and very long	Clear: deposit ..	+	+	+	-	-	+	-	+
81	"	Long and very long	Clear: deposit ..	+	+	+	-	-	+	-	+
82	"	Medium and long	Uniform turbidity	+	+	-	+	-	-	-	+
83	"	Long .. ..	Clear: deposit ..	+	+	+	+	+	+	-	+
84	"	Medium and long	Uniform turbidity	+	+	+	-	+	-	-	+
85	Case I. Two front Q.	Medium and long	Slight turbidity: deposit.	+	+	+	+	+	+	-	+
86	"	Medium and long	Slight turbidity: deposit.	+	+	+	+	+	+	-	+
87	"	Medium and long	Slight turbidity: deposit.	+	+	+	+	+	+	-	+
88	"	Medium and long	Slight turbidity: deposit.	+	+	+	+	+	+	-	+
89	"	Medium and long	Slight turbidity: deposit.	+	+	-	+	-	-	-	+
90	Case I. (40 days later) R. H. Q.	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
91	"	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
92	"	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
93	"	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
94	"	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
95	"	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
96	"	Conglomerate chains.	Clear: deposit ..	-	+	-	-	+	-	-	-
97	"	Separate short chains and inter- lacing chains.	Clear: deposit ..	+	+	-	+	+	-	-	-

TABLE 11—continued.

Number.	Source.	Morphology (length of chains).	Characters of the growth in broth.	Acid in 3 days in							of coagulation milk.
				Lactose.	Saccharose.	Mannite.	Salicin.	Coniferin.	Raffinose.	Inulin.	
98	Case III.	Very long .. ..	Clear: deposit ..	+	+	-	-	+	+	-	+
99	Case IV.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	+	-	+
100	R. F. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	+	-	+
101	Case IV.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
102	R. H. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
103	Case IV.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
104	L. F. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
105	Case IV.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
106	L. H. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
107	Case V.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
108	R. F. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
109	Case V.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	+	-	+
110	R. H. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	+	-	+
111	Case V.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
112	L. F. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
113	Case V.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
114	L. H. Q.	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
115	"	Very long: twisted	Clear: deposit ..	+	+	-	-	-	-	-	+
116	Case VI.	Long: twisted ..	Slight turbidity: deposit.	+	+	-	+	+	-	-	-
117	R. F. Q.	Long: twisted ..	Clear: deposit ..	-	-	-	-	-	-	-	-
118	Case VI.	Long: twisted ..	Clear: deposit ..	+	+	-	+	-	+	-	+
119	L. F. Q.	Long: twisted ..	Clear: deposit ..	+	+	-	+	-	-	-	+
120	"	Very long: twisted	Clear: deposit ..	-	+	-	-	+	-	-	+
121	"	Long: twisted ..	Clear: deposit ..	+	+	-	+	-	-	-	+
122	Case VI.	Short and medium	Uniform turbidity: deposit.	+	+	-	+	-	-	-	+
123	L. H. Q.	Short and medium	Uniform turbidity: deposit.	+	+	-	+	-	-	-	+
124	"	Short and medium	Uniform turbidity: deposit.	+	+	-	+	+	-	-	+
125	Case VI.	Long: very twisted and interlacing.	Clear: + deposit..	-	-	-	-	-	-	-	-
126	R. H. Q.	Long: very twisted and interlacing.	Clear: + deposit..	-	-	-	-	-	-	-	-
127	"	Long: very twisted and interlacing.	Clear: + deposit..	-	-	-	-	-	-	-	-
128	"	Long: very twisted and interlacing.	Clear: + deposit..	-	-	-	-	-	-	-	-
129	"	Long: very twisted and interlacing.	Clear: + deposit..	-	-	-	-	-	-	-	-
130	Case VI. Guinea-pig.	Long: very twisted and interlacing.	Clear: + deposit..	-	-	-	-	-	-	-	-

TABLE 12.

Number.	Source.	Morphology.	Character of broth growth.	Acid in 3 days in							Coagulation of milk.
				Lactose.	Saccharose.	Mannite.	Salicin.	Coniferin.	Raffinose.	Inulin.	
53	Case III.	Diplococci only ..	Uniform turbidity	+	-	+	-	-	+	-	+
54	I. H. Q.	Diplococci only ..	Uniform turbidity	+	-	+	-	-	+	-	+
55	"	Diplococci only ..	Uniform turbidity	+	-	+	-	+	+	-	+
56	"	Diplococci only ..	Uniform turbidity	+	-	+	-	-	+	-	+

TABLE 13.

Number of the cow.	Individual quarters examined.	Number of leucocytes per cubic mm.	Varieties of leucocytes (percentages).				Appearance of the fluid yielded by the different quarters.
			Polymorpho-nuclear.	Lymphocytes.	Large leuco-cytes.	Other kinds.	
I.	Right Hind ..	11,250	.	.	.	.	Turbid brown whey with abundant white clots.
I.	Right Fore ..	18,000	92	2	6	.	Normal milk.
I.	Left Fore ..						
I.	Left Hind ..	19,800	90	3	7	.	Slightly yellowish milk.
I.	Right Hind ..	34,000	93	0	7	.	Brown whey with clots, as before.
I.	Right Fore ..	280	60	5	35	.	Normal milk.
I.	Left Fore ..	1,550	50	18	30	2	Normal milk.
I.	Left Hind ..	180	35	35	30	.	Normal milk.
II.	Right Fore ..	380	65	20	15	.	Normal milk.
II.	Right Hind ..	—	.	.	.	.	—
II.	Left Fore ..	98	50	40	10	.	Normal milk.
II.	Left Hind ..	410	.	.	.	.	Normal milk.
III.	Right Fore ..	196	40	20	40	.	Normal milk.
III.	Right Hind ..	240	35	5	60	.	Normal milk.
III.	Left Fore ..	180	60	10	30	.	Normal milk.
III.	Left Hind ..	9,480	21	4	75	.	Milk containing much blood.
III. (10 days later).	Left Hind ..	3,500	24	23	53	.	Normal milk.
IV.	Right Fore ..	Not estimated.	.	.	.	.	Turbid yellow fluid containing clots.
IV.	Right Hind ..	3,380	.	.	.	.	Milk with a yellow tinge.
IV.	Left Fore ..	Not estimated.	.	.	.	.	A thin blood-stained fluid.

TABLE 13—*continued.*

Number of the cow.	Individual quarters examined.	Number of leucocytes per cubic mm.	Varieties of leucocytes (percentages).				Appearance of the fluid yielded by the different quarters.
			Polymorpho-nuclear.	Lymphocytes.	Large leuco-cytes.	Other kinds.	
IV.	Left Hind ..	1,990					Same as R. F. Q.
V.	Right Fore ..	10,040					Normal milk.
V.	Right Hind ..	196,000					Brown; obviously purulent.
V.	Left Fore ..	12,370					White milk with pus.
V.	Left Hind ..	186,000					Light brown fluid.
VI.	Right Fore ..	1,910	10	15	75		Normal milk.
VI.	Right Hind ..	2,240	60	10	25	5	Turbid yellow white fluid with clots in it.
VI.	Left Fore ..	310	12	45	40	3	Normal milk.
VI.	Left Hind ..	3,360	10	25	65		Normal milk.

*Case II.* A Dutch-bred cow.—Has had seven calves. Calved about five days before the samples were received. Within a day or two of calving the right hind quarter became inflamed, and no milk could be obtained. The inflammation was not severe, and subsided after a short time, the quarter yielding apparently healthy milk.

No fluid could be obtained at all from the affected quarter, but samples were obtained from the three presumably healthy quarters.

This case is only introduced here to show the condition of the other quarters in a case of mild parenchymatous mastitis, affecting only one quarter.

*The cytological conditions present.*—These are shown in Table 13. This table shows that, unlike Case I., the other quarters were not at all involved. Not more than 65 per cent. of the leucocytes were of the polymorphonuclear variety.

*The bacteriological findings.*—0.1 and 1.0 cc. of the milk from each quarter were added to broth and examined in hanging drop preparations after two days' incubation. In no case were streptococci present. Also for each of the three quarters part of the centrifugalised deposit was distributed over an agar plate. The number of colonies were scanty on all the plates, and no streptococci could be isolated.

It being impossible to obtain any fluid from the affected quarter the cause could not be ascertained, but the examination of the other quarters shows that there was not the slightest evidence of their being implicated.

*Case III.* A young cow.—Has had two calves. Calved about nine days before the sample was collected. Almost immediately

after calving the milk from the left hind quarter failed, and none could be obtained from it, although the milk from the other three quarters was quite unaffected. The quarter was scarcely, or not at all, swollen, and there was very little inflammation. When first seen by the veterinary surgeon, five days after calving, no fluid could be obtained, and the fluid obtained nine days after calving was only obtained after passing a sterile tube. There was no disturbance of general health, and the other quarters remained healthy.

Two weeks after calving the milk was said to be normal again, but a sample collected from the affected quarter 19 days after calving still showed a high leucocyte count.

*The cytological conditions present.*—The number of leucocytes and the different kinds are shown in Table 13. The milk of the healthy quarters showed quite low counts, and no evidence of being involved.

The fluid from the affected quarter contained much blood, but otherwise looked like ordinary milk. The number of leucocytes per cubic mm. was high, but their differential enumeration showed that the relative proportion of the different kinds was quite different from that met with in suppurative conditions, and the high count was not due to suppuration.

When re-examined ten days later the inflammation was gradually subsiding, but the large leucocyte (many very large) with a single unsegmented nucleus was still the most prominent cell present.

*The bacteriological results.*—The films prepared from the centrifuged 10 cc. of milk showed no, or practically no, organisms, either from the affected or from the healthy quarters.

Broth tubes were inoculated with 0.1 and 1.0 cc. of the milk from each quarter and examined, in hanging drop preparation, for streptococci, after two days' incubation. Short chains, but no long chains, were fairly numerous in the preparations from the affected and the left fore quarters but were not met with in the other cases.

For the affected quarter 0.1 cc. of the fluid was distributed without re-charging over three agar plates; very numerous colonies developed. By far the most conspicuous and numerous were ordinary white opaque staphylococci, and there were several hundreds of these present. Yellow staphylococcus colonies were also fairly numerous. In addition there were a number of minute colonies, possibly streptococci. A large number of these were subcultivated and their characters determined, but only one of them was a streptococcus. The others were of the translucent staphylococcus type, described as very abundant in healthy milk in the first part of this report. The characters of some of these organisms are shown in Tables 11 and 12.

The deposit from 10 cc., centrifuged for 10 minutes, was for each quarter distributed over agar plates.

The agar plate from the deposit of the right hind quarter showed a good many white and yellow staphylococcus colonies, but only two organisms of the translucent staphylococcus type, and no streptococci. They were much less numerous than from the

affected quarter. The plates from the deposits from the other healthy quarters showed very few colonies.

A number of inoculations were performed. A guinea-pig injected intraperitoneally with 2.5 cc. of the milk from the affected quarter showed no ill effects, while a mouse fed with the centrifugalised deposit from 10 cc. of the same quarter also remained well. A mixture of broth cultures of two of the diplococci (*i.e.*, Nos. 54 and 55) injected intraperitoneally into a mouse was non-pathogenic. Another (No. 56) injected subcutaneously also produced no ill effects, while the only streptococcus isolated (No. 98) also was non-virulent when injected subcutaneously into a mouse.

The cause of the slight inflammatory condition was evidently not due to streptococci, and whatever the cause it was of a slight grade and more or less transitory.

*Case IV.* A roan shorthorn cow.—Not in calf. Calved about six months before the onset of the mastitis. The inflammation started three weeks before the samples were collected in the right fore quarter, which was at first noticed to be slightly enlarged, but yielding apparently unaltered milk. The milk, however, soon became wrong in this quarter, then the right hind quarter became involved, and subsequently the left hind, and lastly the left fore quarter.

The cow stood with others in a shed, but the other cows did not become affected.

The characters of the fluid from the different quarters when the samples were collected are indicated in Table 13.

*The cytological conditions present.*—In a case of this kind these are unimportant.

The number of leucocytes was not estimated in two of the fluids. In the quarter yielding a milk-like fluid (right hind) the number of leucocytes was 3,360.

*The bacteriological findings.*—The centrifugalised deposits from the different quarters showed in every case the presence of very numerous long twisted chains of streptococci, and in great abundance. They were particularly abundant in the right hind quarter.

To isolate the bacteria present small quantities of the fluid from each quarter were distributed over a series of agar plates. A few staphylococcus colonies were present, but the vast majority of the colonies were those of streptococci. These were especially numerous in the two hind quarters, the plates being thickly covered with such colonies. Two from each quarter were culturally worked out. Their characters are given in Table 11. This table shows that the eight streptococci were absolutely identical, except that two of them fermented raffinose with acid production.

The animal inoculation results are also of interest. A guinea-pig injected intraperitoneally with 1.5 cc. of fluid from the left hind quarter remained apparently well, but developed a local abscess at the site of inoculation. It was killed 18 days after inoculation, a few days after the abscess was first recognized. No internal lesions, apart from the abscess, were noticed

while cultivations from the spleen remained sterile. From the abscess, on the other hand, a streptococcus forming long chains and culturally indistinguishable (except that it fermented salicin) from the streptococci isolated from the different quarters was obtained. A few colonies of a translucent staphylococcus forming quite small groups of cocci in broth and coagulating milk within three days were also present, and a very few white staphylococci. Otherwise the long chain streptococcus was present in pure culture.

A mouse injected intraperitoneally with the centrifuged deposit of 10 cc. of the right hind quarter showed no ill effects and no abscess developed. A mouse fed with the deposit of another 10 cc. of the same quarter also showed no ill effects. A mouse injected with 0.5 cc. of the fluid from the right front quarter also remained apparently quite unaffected.

The injection of pure cultures of the bacilli isolated were also without ill effects. Thus mice injected intraperitoneally with streptococcus 99 and a mixture of streptococci 101, 102, and 105 remained well. Also mice injected subcutaneously with one of the translucent streptococci and an ordinary white staphylococcus isolated from the agar plates remained unaffected.

The bacteriological results show that the cause of this mastitis was almost certainly the long chain streptococcus, an organism abundant in every quarter, but of low virulence to mice and guinea-pigs.

*Case V.*—A roan shorthorn cow; has had 3 or 4 calves. Purchased freshly calved, and on the day following both the hind quarters became hard and enlarged. This increased, although not painful and the milk became discoloured, and contained pus. When the samples were collected 12 days after the onset all four quarters were affected.

There was no rise in temperature and the general health of the cow was apparently unaffected.

No other cows were subsequently attacked.

*The cytological conditions present.*—These are shown in Table 13. The findings show the very large number of leucocytes present in cases which are obviously purulent to the naked eye. For the most part the leucocytes were much broken up and a satisfactory differential count was impossible.

*The bacteriological results.*—The examination of the fluid itself without any centrifugalisation showed for all four quarters long streptococcus chains in great abundance. They were the only organisms found, and were very readily shown in microscopic preparations made by spreading one loopful of the fluid on a coverslip and staining by methylene blue.

One loopful of the fluid from each of the quarters spread over agar plates showed innumerable minute translucent colonies which on subcultivation were streptococci. They were present in practically pure culture, and were equally abundant in the fluid from all four quarters. Two typical colonies from each quarter (three from the left hind) were culturally worked out. Their characters are given in Table 11, and, except that two of them

fermented raffinose, they were all absolutely identical. They were identical with the streptococci of Case IV.

The animal inoculation results were very similar to those of Case IV. A guinea-pig injected intraperitoneally with 2 cc. of fluid from the left hind quarter showed after two weeks a well marked abscess at the site of inoculation. The animal was killed 16 days after the inoculation. Post-mortem—apart from the abscess—no lesions were noticed, while cultivations from the spleen remained sterile. From the abscess, which contained caseous solid white pus, a streptococcus was isolated in quite pure culture, and which in its characters was quite identical with the streptococci isolated from the different quarters.

A mouse injected intraperitoneally with the 10 cc. centrifugalised deposit of the right hind quarter showed no ill effects.

A mouse fed with bread soaked in the left fore quarter fluid remained unaffected, as did also a mouse injected subcutaneously with the fluid from the left hind quarter.

Mice injected intraperitoneally and subcutaneously with the isolated streptococci remained quite unaffected.

On the other hand, a guinea-pig injected intraperitoneally with a mixture of agar emulsions of two of the isolated streptococci (Nos. 109 and 113) showed itself distinctly ill two days later and died three days after the inoculation. Post-mortem—there was a local abscess at the site of injection, but otherwise no macroscopic lesions. Cultivations from the spleen, peritoneal fluid, liver and heart blood showed no growth. No cultivations were made from the abscess.

The bacteriological results show that the cause of this mastitis was a long chain streptococcus identical with that causing the mastitis in Case IV.

*Case VI.*—A red shorthorn; has had three calves. The cow had calved just over a week before the samples were collected. A valuable cow which for months had been giving 23 quarts of milk a day.

When the samples were collected the right hind quarter alone was said to be affected. The udder was enlarged and this quarter was somewhat indurated. Mr. Shaw, who kindly supplied me with the samples, informed me nine days later that the left hind quarter had become indurated and was yielding a purulent milk.

The general health of the cow was unaffected.

The fluid from the right hind quarter was turbid and yellow-white with numerous clots in it.

*The cytological conditions present.*—These are of considerable interest in that a high count was obtained with the left hind quarter milk from a quarter which at the time was apparently unaffected but which nine days later had become distinctly involved.

Only in the affected quarter did the proportion of the different kinds of leucocytes approximate to the conditions met with in purulent cases.

*The bacteriological results.*—The examination of preparations made from the 10 cc. centrifugalised deposits of the two fore quarters showed no bacteria. On the other hand, the deposit of

10 cc. of the left hind quarter showed exceedingly numerous quite short streptococci, but no very long chains. The centrifuged deposit of 2 cc. from the right hind quarter showed a few short, but no long chains. One loop of fluid from this quarter added to broth, after 2 days' incubation examined in hanging drop preparation, showed long twisted streptococcus chains in apparently pure culture.

0.1 and 1.0 cc. of the fluid from the other quarters added to broth tubes showed no streptococci (in hanging drop preparation) in the cultivations from the right fore quarter, but streptococci of various lengths were fairly numerous in those made from the left fore and left hind quarters.

No glucose fermenters were present in 10 cc. of fluid from any of the quarters.

One loopful of fluid from the affected quarter spread over an agar plate showed next day a very abundant growth of streptococcus colonies in practically pure culture. A number of these were sub-cultivated and 5 of them fully worked out (*see* Table 11) showed identical characters.

Portions of the centrifuged deposit of 10 cc. from the other quarters were distributed over agar plates. All the plates showed abundant minute colonies which on investigation showed themselves to be those of streptococci (*see* Table 11).

A number of animal inoculation experiments were performed.

A guinea-pig injected intraperitoneally with 1.5 cc. of the fluid from the affected quarter (R. H. Q.) died three days later. Post-mortem—the whole abdominal and thoracic musculature was deep red and thickened with a considerable blood stained exudation.

Microscopic preparations of the stained exudation showed very numerous cocci either single or as diplococci. The peritoneal fluid was slightly in excess, and there was evidence of peritonitis. Diplococci were fairly numerous in preparations made from the heart blood. Otherwise there were no obvious macroscopic lesions. From the peritoneal fluid, spleen, heart blood and liver, the same organism (streptococcus No. 130, Table 11) was isolated in pure culture, an organism identical with those isolated directly from the fluid yielded by this quarter.

A mouse injected subcutaneously with 0.5 cc. of fluid from the affected quarter died in two days. The spleen was enlarged and of a dark colour and an organism identical with that obtained from the guinea-pig was isolated from the spleen, liver, and heart blood. A mouse *fed* with the same fluid upon bread showed no ill effects.

A mouse injected intraperitoneally with the deposit of 10 cc. of the left hind quarter milk showed no ill effects.

A number of mice were also injected with broth cultures of isolated streptococci.

A mouse inoculated subcutaneously with streptococcus 127 died in six days. Post-mortem—the animal showed extensive exudation under the skin of the abdomen with an enlarged dark spleen. The inoculated organism was recovered in pure culture from the spleen and heart blood.

Streptococci 116, 118, 122, and 123 all from one or another of the other three quarters when inoculated subcutaneously in the same way were non-virulent.

In this case the cause of the mastitis was evidently a long chain streptococcus of well-marked virulence to guinea-pigs and mice. This streptococcus was, however, quite distinct from that causing the two previous udder inflammations.

The fluid from the other quarters while containing abundant streptococci yet were non-virulent and contained streptococci of quite different characters and pathogenicity.

### SECTION III.

#### *Comparison of the different Cases with one another.*

Of the five cases examined (excluding No. II., in which the affected quarter fluid was not received), in three, streptococci were certainly the cause, and in a fourth the probable cause, while in the remaining case, although the infective agent was not ascertained, it was not due to a streptococcus infection.

Of the four streptococci isolated only two were identical. Only in these two cases were all four quarters involved.

The question as to how far other quarters were involved is of importance. In my experience it is the frequent, if not the usual practice, to allow the milk from the other quarters, when only one is apparently affected, to go into the milk pail and to be sold with the rest. For this reason careful examination was made, in every case, of all four quarters in order to ascertain how far they were affected, and to what extent the organisms causing the mastitis were present in milk which to the naked eye appeared normal or sufficiently normal to be passed by the milker as healthy milk.

In Cases IV. and V., in which all four quarters were affected, in all four the causal organism was found, but it should be noted that the milk from the right hind quarter of No. IV. and the right fore quarter of No. VI. would be passed by a milker as healthy milk.

In Cases I. and VI., in which only one quarter was involved, the causal streptococci were only found in the affected quarters. It is of interest, however, to note, particularly for No. I., that the number of leucocytes present in the other quarters was greatly increased, and certainly pus was present in the milk.

A comparison of these streptococci with those found in the individual cows examined in Part I., and with streptococci pathogenic to man, is instructive.

The streptococcus from the affected quarter of No. VI. is a highly pathogenic form, quite unlike any of the streptococci isolated from the healthy milk samples. In addition to the characters given in Table II, it retains the stain by Gram's method, it grows, but not readily, upon gelatine at 21° C. with the ordinary streptococcus-like growth and without liquefaction.

It produces very little acid in litmus milk. With the saccharose and raffinose media a little acid was sometimes produced, but never a clear acid reaction.

Nothing quite identical with the streptococcus No. 72, and similar organisms from the affected quarter of Case I., were found in any of the healthy milk samples, but streptococci only differing in the fermentation of one or other sugar-alcohol were not uncommon. A comparison of the characters of the two streptococci from Cases IV. and V., with the streptococci described by Nocard and Mollereau in 1886, and isolated by them from the milk of cows suffering from chronic mastitis, shows that they are the same organism. The organism is apparently the same as the *Streptococcus mastitis contagiosæ* of Guillebeau.

In addition to the characters given in Table 11, these organisms retained the stain by Gram's method, grew at 21° C. upon gelatin slope, producing a delicate translucent growth without liquefaction, grew well under anaërobic conditions and grew rapidly upon agar slope as a translucent growth of small discrete circular colonies.

Records as to the action of Nocard and Mollereau's streptococcus upon the sugar-alcohols are not available, but in their other characters the agreement is exact.

Since these streptococci play an important part in the etiology of mastitis, it is interesting to compare them with those found in the milk samples from the individual cows.

The streptococcus found in the cows with ulcerated teats, and in a few cases without any local lesions is very similar in character to this form. The chief difference is in regard to the fermentation of salicin and raffinose. The mastitis streptococci never fermented salicin (when isolated from the fluid direct), and only four out of seventeen fermented raffinose. Otherwise their action to Gordon's tests and to the other tests employed was identical.

The reaction with salicin is one upon which, in my opinion, too much reliance should not be placed. These acid fermentation tests were, as a rule, stable, but I have occasionally found organisms which produced no acid with salicin, but on re-examination showed well marked acid production.

As previously mentioned the streptococcus isolated from the guinea-pig injected with the fluid from Case IV. was culturally indistinguishable from the streptococci so numerous in the injected fluid, except that it fermented salicin.

It would seem then that the organisms of these two cases of mastitis approximate closely in their characters to the long chain forms found not infrequently in milk. With organisms of this class, and in a preliminary report dealing with only a few cases, it is not justifiable to press the question further, but it at least suggests the possibility that some cases of mastitis are due to streptococci more or less naturally present, or if not naturally present then present without causing marked inflammation, which, owing to some damage to the udder (such as lying upon damp grass, local injury, &c.), have their virulence sufficiently exalted to enable them to set up local inflammation in the udder. With present evidence, however, this is but a working supposition.

Of even greater interest is the possibility of these streptococci being related to the streptococci associated with definite pathological effects in man.

At present not much can be said, but it is of interest to note that the type of streptococcus to which the streptococcus of Cases IV. and V. closely approximates is that described by Andrewes and Horder in a valuable paper on this group ("A Study of the Streptococci Pathogenic for Man," "Lancet," September 15th, 22nd, 29th, 1906), as the *Streptococcus anginosus*.

Its essential characters are that it is a long-chain form, producing a flocculent deposit in a clear broth, which clots milk and reduces neutral red.

It frequently will not grow upon gelatin at 20° C. Lactose and saccharose are fermented, and raffinose not infrequently; the others are not fermented.

Apart from the neutral red reaction, which was not tested, the streptococci of these two cases of mastitis agree in their characters with this *Streptococcus anginosus*.

Andrewes and Horder describe this type as frequent in sore throats, and as having a special connection with inflammation of the fauces and with scarlet-fever.

In view of the strong epidemiological connection established between certain specific cases of mastitis and outbreaks of sore throat, this close relationship is certainly striking, and suggests further and more definite investigation. Particular stress has been laid upon the streptococcus obtained from cases IV. and V., since this organism is apparently identical with the streptococci isolated from cases of mastitis, by Nocard and Mollereau, Lucet, Guillebeau, Hess and Borgeaud, Steiger, and other Continental workers.

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## APPENDIX B., No. 5.

## REPORT on the DISTRIBUTION of ORGANISMS of the GAERTNER GROUP in the ANIMAL INTESTINE; by WILLIAM G. SAVAGE, M.D.

The isolation of members of this group from intestinal contents or other substances rich in bacterial life is essentially a matter of good technique. In the intestinal canal, if they are present, they will invariably be associated with *B. coli*, the latter usually being in vastly superior numbers; and unless reliable methods are available whereby their colonies can be satisfactorily differentiated from those of *B. coli*, they will probably be overlooked.

Before, therefore, any work can be considered dealing with the distribution of this class of organisms, it is necessary to investigate how far any known methods of isolation are sufficiently delicate and reliable, and the best method or methods to employ. The work done in regard to this group in the present report is considered in two parts.

PART I.—An inquiry into the most suitable methods for the isolation of the Gaertner group of organisms.

PART II.—A report upon the results of an examination of the intestinal contents of certain presumably healthy animals.

The importance of this group in relation to public health is very great, since the group includes the chief bacteria of meat poisoning and the organisms of hog-cholera and paratyphoid fever.

The available information is very scanty as to the immediate sources of infection for the diseases caused by this group of bacteria. Thus, for example, the *Bacillus enteritidis* of Gaertner has been isolated as the cause of many outbreaks of food poisoning, but definite information as to how the meat became infected and the primary source of the bacilli is almost always lacking.

In the same way for the different cases of paratyphoid fever, members of the Gaertner group have been shown to be the infective agents, but we have little information as to the source of infection.

It is a not unreasonable supposition that the source of infection is excrementitious matters, either from a healthy bowel or from one specifically infected, a supposition which a study of the recorded outbreaks supports.

Thus, for example, in the extensive outbreak of food poisoning in Derby in 1902, the cause of the outbreak was ascertained to be the *Bacillus enteritidis*, and the results of the inquiry of Professor Delépine and Dr. Howarth led them to the view that "the presence of the *Bacillus enteritidis* in the pies was due to faecal pollution of the meat, before it was baked."

In a case of paratyphoid fever in Colchester, the only source of infection which seemed at all likely was that the patient, a pork-butcher working in a dirtily kept shop, became infected in connection with his work.

A consideration of the literature of the infections due to this group of organisms suggests that an investigation of the contents of animal excreta is likely to be of value in relation to the etiology of these diseases.

## PART I.

### *An Inquiry into the most suitable Methods for the Isolation of the Gaertner Group of Organisms.*

The Gaertner group comprises a number of different organisms, but most of them are culturally identical, their differentiation being based upon a study of their capacity to be agglutinated by specific sera obtained by immunising animals with different members of the group.

In their cultural characters they stand between the typhoid and the *B. coli* groups. They resemble the former, amongst other characters, in that they do not ferment lactose, differing in this way from most members of the *B. coli* group.

The methods likely to be of value for the isolation of members of this group from excreta, intestinal contents, &c., fall naturally into three divisions.

- I. Direct plating methods.
- II. Enrichment methods.
- III. Animal inoculation methods.

#### *Direct Plating Methods.*

Direct plating over solidified gelatine or agar is useless, since these media are quite inadequate to differentiate the colonies of members of this group from those of *B. coli*.

Since *B. coli* ferments lactose while members of this group do not, the special media containing lactose designed to differentiate lactose from non-lactose fermenters are especially suitable. Four of these may be mentioned.

- (a) The well-known *Drigalski-Conradi agar* yields *B. coli* colonies which are red and non-translucent, while the *B. gaertner* colonies are blue, more translucent and smaller. I employed this medium extensively in 1904-1905 to isolate members of the Gaertner group from excreta and intestinal contents, but found it inferior to the media here recommended.
- (b) *Malachite Green Agar*.—In view of the favourable results obtained with the two following media this agar was not experimented with, but it has been strongly recommended in Germany as suitable for the isolation of members of this group.
- (c) *Lactose Neutral-red Bile Salt Agar*.—This medium is a modification by Grünbaum and Hume of MacConkey's

well-known bile salt agar. Upon this medium *B. coli* colonies are bright red, while those of *B. gaertner* and other non-lactose fermenting organisms are white. When the plates are not overcrowded the distinction is very sharp.

- (d) *Fuchsin-agar*.—This medium, introduced by Endo in 1904, is a fuchsin lactose agar decolourised by sodium sulphite. The distinction between the colonies is also very marked with this medium, the *coli* colonies being bright red, the non-lactose fermenting organisms, such as the typhoid bacillus and members of the Gaertner group, colourless or a faint pink.

A number of experiments were carried out by brushing mixtures of either *B. coli* and a member of the Gaertner group, or excreta inoculated with a Gaertner bacillus, over plates containing solidified Drigalski-Conradi agar, fuchsin agar (modified as described below), and neutral-red bile-salt agar.

Both the latter gave clearly defined and sharply differentiated colonies and were found to be very suitable for routine work. Both media can be readily prepared and of uniform and regular composition and appearance.

The fuchsin-agar when made up according to the original directions was not found to give good results, but after careful testings the following modified medium was found to be satisfactory, and was employed in the different experiments and examinations described below. The chief modifications are in regard to the amount of fuchsin and in the preparation of a medium which shows a constant composition.

*Modified fuchsin-agar*.—Method of preparation :—

Peptone, 10 grammes; Liebig's extract of beef, 10 grammes; sodium chloride, 5 grammes, are boiled up in an enamelled dish with 1 litre of distilled water. The mixture is then poured into a flask, 30 grammes of powdered agar added, and the whole heated in the autoclave at 115° C. for one hour. The flask is removed, and, after cooling to about 60° C., the white of one egg mixed with a little distilled water is added. The contents are coagulated by heating in current steam in the usual way, filtered, and the filtrate made up to 1 litre. The mixture is made neutral, litmus paper being used as the indicator. Then 19 cc. of *normal* sodium carbonate solution and 10 grammes of chemically pure lactose are added. The flask is replaced for 30 minutes in the steam sterilizer. Almost invariably there is a considerable precipitate and the mixture has to be again filtered.

Seven cubic centimetres of the fuchsin solution (see below) are added, followed by 25 cc. of a quite freshly prepared 10 per cent. sodium sulphite solution. The mixture becomes much less red, but is not immediately decolourised. It is then tubed, conveniently into small flasks each containing 50–60 cc. of media, and sterilized in current steam for two days, 30 minutes each day.

The *fuchsin solution* is made as follows :—3 grammes of powdered crystalline fuchsin are placed in a dry flask and 60 cc. of absolute alcohol are added. The contents are thoroughly well mixed and the flask, tightly stoppered, allowed to stand for

exactly 24 hours at 20-22° C. The alcoholic extract is then decanted and preserved in a clean glass-stoppered bottle. Made in this way a uniform fuchsin extract is obtained which keeps well, and the same quantity of fuchsin is added each time a fresh batch of medium is prepared, a matter of much importance.

As described by Endo, 5 cc. of a 10 per cent. fuchsin solution in 96 per cent. alcohol is to added, but very different strengths of fuchsin will be obtained according as to whether heat is used to make the solution, the time the alcohol and fuchsin are allowed to stand together, &c. Also in my experience this proportion of fuchsin is too high.

The medium must be stored in the dark since light gradually turns it red. When solidified it is almost free from colour.

The lactose neutral-red bile-salt agar was made up in the ordinary way, enough for four plates being sterilized in each flask. One point is of importance in the use of these media. To obtain satisfactory discrete colonies it is necessary that the plates should be thoroughly dried. The procedure adopted was rapidly to solidify the media in the Petri dishes, using a plate-cooling apparatus, distribute the inoculated substance over them by means of a bent sterile glass rod in the usual way, and then dry the inoculated plates in the 37° C. incubator *uncovered* for 1 to 2 hours. The covers were then replaced, the plates inverted, and the incubation continued.

#### *Enrichment Methods.*

As relatively few bacilli of the Gaertner group are likely to be present in intestinal contents compared with the vast numbers of *B. coli*, some method whereby the members of the former group can be relatively increased is very desirable. The ideal enrichment method is one which allows the Gaertner bacilli to multiply while the *B. coli* organisms are restrained from growing or, at least, retarded.

The chief members of this group all ferment dulcitol, although it cannot at present be positively affirmed that this is a universal characteristic of the group.

It is a fact of very general application that organisms which ferment different sugars and alcohols tend to proliferate in suitable media containing those particular substances to a greater extent than allied similar organisms which do not ferment these bodies. I made extensive use of this property in 1903-4 while investigating the distribution of different kinds of *B. coli* in human and animal excreta, and Boycott (Journal of Hygiene, Vol. VI., p. 33, 1906) has recently drawn attention to it in connection with the dulcitol fermentation properties of the Gaertner group.

Boycott in the paper referred to above recommends the use of dulcitol bile-salt peptone-water, a medium introduced by MacConkey. This medium was made the subject of investigation.

The use of malachite green in the form of malachite green agar was introduced by Loeffler in 1903. It was modified by Lentz and Tietz and others, and extensively used for the isolation of the typhoid bacillus from excreta. It was also recommended as valuable for the isolation of the paratyphoid bacillus,

It was thought desirable therefore to study the value of malachite green as an addition to liquid media, to ascertain if it could be employed to restrain *B. coli* organisms without preventing the growth of Gaertner organisms thus ensuring a proportionate proliferation of the latter.

In the experiments detailed below the malachite green was added to broth media. And in view of the action of members of this group upon dulcete this substance was also added to the broth. Some experiments of Klinger have shown that the reaction of the medium is of importance in investigating the restraining power of malachite green, and he found that a reaction of + 1 per cent. was the most suitable. The medium was always made up to an accurate + 1 per cent.

The method of preparation of the dulcete malachite green broth (0.05 malachite green) is as follows:—

Liebig's extract 10 grammes, peptone 10 grammes, sodium chloride 5 grammes, are boiled up with a litre of distilled water. The mixture, after filtration, is made up accurately to a + 1 per cent. reaction, and 5 grammes of dulcete are added. 0.5 gramme of powdered malachite green is very accurately weighed out and also added. The mixture, usually slightly turbid, is steamed for 30 minutes and again filtered. It is tubed, 10 cc. into each tube, and sterilized for 30 minutes on two successive days. It is of course important to see that all the malachite green is dissolved before the second filtration.

Caffeine has also been credited with a restraining action upon *B. coli* while not inhibiting the growth of *B. typhosus*. It was thought possible that it might be useful for the isolation of Gaertner organisms, but a few preliminary experiments with caffeine broth did not show satisfactory results, and the use of caffeine was not further investigated.

In view of the satisfactory results obtained with the enrichment fluids employed, it was not considered necessary to study further media of this kind.

#### *Animal Inoculation Methods.*

As a class the Gaertner group of organisms are possessed of a high degree of virulence for rodents, a virulence as a rule greatly in excess of that shown by most *B. coli*, derived from the animal intestine.

Injection of the excreta, or other material under examination, either direct or after preliminary incubation in a suitable medium, would, therefore, seem a likely aid toward their isolation, the spleen and other internal organs showing the presence of such bacteria either in pure culture or sufficiently numerous for ready identification. Such a method has been extensively employed by Morgan (British Medical Journal, June 10th, 1905), and with valuable results.

There are, however, several practical objections to this method. Firstly, while it is true that the organisms of the Gaertner group are, as a class, more pathogenic than those of the *B. coli* group, yet this is not true for all the individual members. Many *B. coli*

from animal excreta are pathogenic; and, in particular, if broth or other liquid medium is inoculated with excreta and injected, after a preliminary incubation, a pathogenic result is very likely to accrue due entirely to members of the *B. coli* group. Results showing this were obtained by Houston (Report of the Medical Officer, Local Government Board, 1903-4, p. 483) with guinea-pigs injected with broth cultures which had been inoculated with minute amounts of human fæces.

In the presence of highly virulent members of the *B. coli* group the pathogenic effects may be due entirely to members of this group, and even if a few Gaertner organisms had been present they may have been overgrown or suppressed.

Secondly, it is a quite feasible supposition that Gaertner group organisms may be present but non-virulent. In this case this method of isolation would be useless.

Thirdly, a more important objection is that it cannot be considered an altogether reliable deduction to assume that because members of this group were isolated from the internal organs of an animal recently inoculated, the Gaertner organisms isolated were contained in the materials used for the inoculation. For example, MacConkey (Journal of Hygiene, V., p. 343, 1905) obtained bacteria of this group from the heart blood of guinea-pigs inoculated with *B. mallei* and with *B. typhosus*.

Also Smallman (Journal Royal Army Medical Corps, Vol. V., p. 137) injected over 200 guinea-pigs intraperitoneally or subcutaneously with either living or dead typhoid bacilli. In 22 instances (about 11 per cent.) organisms of the Gaertner group were obtained. In both these sets of experiments the Gaertner group organisms were certainly not contained in the materials inoculated.

From this point of view the results of some experiments and inoculations of my own upon mice, recently undertaken in connection with the bacteriology of milk, which are detailed in another part of this report, are of interest. In several instances Gaertner or coli group organisms were isolated in pure culture from mice which had been inoculated with streptococci or other milk bacteria. The animals were usually found dead when the laboratory was opened in the morning, and the post-mortem examination was made at once.

The details of these experiments are, very briefly, as follows:—

*Case 1.*—Mouse injected subcutaneously with 0.5 cc. of fluid from a case of mastitis in a cow. Died six days later. An organism of Coli type, MG<sub>1</sub> of Table I., isolated in pure culture from the spleen and heart blood.

*Case 2.*—Mouse injected subcutaneously with a mixture of two streptococci. Found dead next day, and a coli-like organism MG<sub>2</sub> of Table I. isolated in pure culture from the spleen, but not from the heart blood.

*Case 3.*—Mouse injected subcutaneously with a mixture of two staphylococci. Found dead three days later, and a Gaertner group organism MG<sub>3</sub> was isolated in pure culture from the enlarged dark spleen and heart blood.

*Case 4.*—Mouse fed (on the same day as mouse 3 was injected) with fluid from a healthy quarter of a cow, one of the

other quarters of which was affected with a mild attack of mastitis. Died six days later. The spleen was enlarged and dark, and an organism MG<sub>4</sub> was isolated in pure culture from it.

- Case 5.*—Mouse fed, on the same day as case 4, but with the milk from another healthy quarter of the same cow. Kept in a separate cage. Died six days later, and an organism MG<sub>5</sub> was isolated in pure culture from the enlarged congested spleen.

The milk of both these quarters was free from glucose fermenting organisms (in 10 cc.) so that neither of the isolated bacilli was derived from the milk used for the feeding.

- Case 6.*—One batch of one dozen mice received from London were evidently diseased when they reached the laboratory, since within a few days they began to die. A careful post-mortem examination was made of two.

In both the spleen was greatly enlarged and very dark. In one the liver was obviously diseased. MG<sub>6</sub> and MG<sub>7</sub> were isolated in pure culture from the heart blood and spleens of the cases respectively.

- Case 7.*—Over two-and-a-half months after these last cases some mice which had either been fed with milk or subcutaneously injected, all over a month previously, and which had subsequently remained apparently unaffected, were placed together in one cage which had been recently sterilized. One of the mice died, or was killed by the others, and in the morning it was found dead and partially eaten. Subsequently the eight mice in this case all sickened and died in succession.

A post-mortem examination on one of them showed an organism MG<sub>8</sub> in pure culture from the spleen, which, however, was not enlarged. Evidently the mouse which first died was infective, and the other mice became infected from it. Presumably MG<sub>8</sub> was the cause of the outbreak.

- Case 8.*—An organism G<sub>3</sub> isolated from a healthy bullock was injected subcutaneously into a mouse. The animal died 17 days later. Post-mortem nothing abnormal was found, and the spleen was not enlarged. From the spleen an organism MG<sub>9</sub> was isolated in pure culture.

From the point of view under consideration this case is of peculiar interest since both the organism isolated and the organism recovered were Gaertner group organisms, which fermented glucose but not lactose, and which produced marked alkalinity in milk. Indeed, at first I assumed them to be identical, but the organism injected did not ferment dulcitol, while the organism recovered readily fermented that substance. Also their reactions to immune sera are quite different. In addition G<sub>3</sub> will not grow in 0.05 malachite green broth, while MG<sub>9</sub> grows readily.

The cultural characters of these isolated organisms are given in Table I. The reaction to different sera of several of these organisms is considered subsequently.

TABLE I.

—	MG <sub>1</sub>	MG <sub>2</sub>	MG <sub>3</sub>	MG <sub>4</sub>	MG <sub>5</sub>	MG <sub>6</sub>	MG <sub>7</sub>	MG <sub>8</sub>	MG <sub>9</sub>
Motility .. ..	+	+	+	+	+	+	+	+	+
Glucose fermentation.	+	+	+	+	+	+	+	+	+
Lactose fermentation.	-	-	-	+	+	-	-	-	-
Neutral red reaction.	+	+	+	+	+	+	+	-	+
Litmus milk ..	Acid and coagulated.	Acid and coagulated.	Acid then alkaline.	Acid and coagulated.	Acid and coagulated.	Acid then alkaline.	Acid then alkaline.	Acid then alkaline.	Acid then alkaline.
Liquefaction of gelatine.	-	-	-	-	-	-	-	-	-
Dulcitol fermentation.	-	-	-	-	-	+	+	-	+
Saccharose fermentation.	-	-	-	-	-	-	-	-	-
Indol production	-	+	-	+	+	-	-	+	-
		little.		little.					

In addition to the above characters they are all short bacilli with rounded ends growing upon gelatine slope media as a bluish translucent growth.

The results in these cases are of considerable interest. MG<sub>5</sub> and MG<sub>7</sub> are identical, and it may be assumed that the outbreak amongst that batch of mice was due to this organism. This bacillus is certainly one of the Gaertner group, and its reactions towards immune sera show it to be identical or allied to the Aertryke group.

It might be thought that the other cases were due to an extension of this outbreak, but some of the cases were before and some a long time afterwards, while the majority of the isolated organisms were culturally quite distinct. MG<sub>3</sub> is very similar, but does not ferment dulcitol.

All the inoculated or fed mice were kept in quite separate cages.

The results show that spontaneous infection of mice with coli or Gaertner group organisms may occur not infrequently, and that caution must be exercised in the use of mice for experimental work. Up to the present I have not met with such spontaneous infection amongst guinea-pigs or rabbits.

These considerations show that the animal inoculation method cannot be relied upon for trustworthy results, and it has not been employed in any of the investigations described in this report.

#### *Experiments with Enrichment Media.*

To test the value of malachite green and other substances as reliable enrichment media, a number of direct experiments were made. In my opinion in carrying out experiments upon the suitability of any particular isolation method too little attention is usually paid to the actual number of organisms added or present in the experiments. The experimental conditions do not sufficiently nearly approximate to those likely to be met with under natural conditions.

For example, in testing the reliability of any given method to isolate typhoid bacilli from water, &c., a not infrequent procedure is to add a loopful of a typhoid broth culture to a few litres of water and then, if the organism is isolated, to claim credit for the

method. A loopful of a broth culture of *B. typhosus* may contain 50,000 or more organisms, so that from a practical point of view deductions from such experiments are quite unreliable since the number of typhoid bacilli added to the water, or other fluid inoculated, is vastly too numerous and quite out of proportion to the numbers likely to be met with under natural conditions, the only conditions for which such a method is required.

It is, therefore, very important in testing methods of this class to approximate to the conditions of practical work, and some idea of the actual number of organisms added should be known and stated in the report. As far as possible this has been done in the following experiments. The source of the different members of the Gaertner group and of the *B. coli* used in the following experiments, and also for the immunisation of the rabbits, was as follows:—

Schotmüller A and B, Aerttryke, Brion and Kayser, *B. psittacosis*, Gaertner D were kindly sent to me by Dr. MacConkey of the Lister Institute, who also supplied me with cultures obtained by him from a small outbreak of food poisoning in Fulham (Journal of Hygiene, 1906, Vol. VI., p. 579) derived from a baby who succumbed to the infection, and from the incriminated rabbit. They are called here MacConkey C and MacConkey R respectively.

Gaertner U.C.H. came from University College, London, source unknown. Paratyphoid B was isolated by me from a case of paratyphoid fever in Colchester in 1904.

The *B. coli* strains were isolated from excreta. (d +), (d —), indicates whether the variety ferments dulcitol or not.

**Experiment I.**—To test the value of dulcitol bile-salt peptone-water as an enrichment medium. A diluted fraction of a 24 hours broth culture of paratyphoid B was added to a tube of dulcitol bile-salt peptone-water, approximately, equal amounts being added to each. In the same way, as far as possible, an equal quantity of a diluted 24 hours broth culture of *B. coli* (d +) was added to the same tubes.

The number of *B. paratyphosus* and *B. coli* in each cc. of these tubes was determined. The tubes were incubated at 37° C. for 23 hours, and their relative distribution again ascertained. To determine the number of organisms definite quantities of the broth or other medium were removed, suitably diluted and an accurately known fraction distributed evenly over suitable solid media.

In this experiment L.B.A.\* plates only used.

The percentage presence of these two organisms at the start and after 23 hours' incubation was as follows:—

	Broth.		Dulcitol peptone water.	
	At start.	After 23 hours.	At start.	After 23 hours.
<i>B. paratyphosus</i> ... ..	37	3	39	85
<i>B. coli</i> (d +) ... ..	63	97	61	15

\* To save space the terms L.B.A. and F.A. are used to signify lactose bile-salt neutral red agar and modified fuchsin agar respectively.

These results show that at the start the *B. coli* were nearly twice as numerous as the paratyphoid bacilli, but that after 23 hours' incubation they formed 97 per cent. in the broth, but only 15 per cent. in the dulcitate peptone water, showing that in the latter the proliferation of the paratyphoid bacilli had been relatively greater, and this although the *B. coli* also fermented dulcitate.

As mentioned above, it is important not to add an excessive number of organisms. This was carefully attended to, and the estimated number of bacilli at the start of the experiment were only—

In 1 cc. of broth, 807 coli and 470 paratyphoid.

In 1 cc. of dulcitate peptone water, 650 coli and 410 paratyphoid.

In both media extensive multiplication had taken place. The dilutions were made as accurately as possible so that an approximate computation could be arrived at. After 23 hours there were—

In 1 cc. of the broth, 350 million coli and 8 million paratyphoid B.

In 1 cc. of the dulcitate peptone water 59 million coli and 353 million paratyphoid B.

These figures are introduced to show that there was no question of retardation or inhibition of either organism, for both multiplied extensively.

*Experiment II.*—To investigate the most suitable strength of malachite green to be employed.

Malachite green dulcitate broth was used prepared as described above, except that three strengths of malachite green were used, i.e., 0.1, 0.05, and 0.025 per cent. malachite green respectively.

The experiment was repeated, but with different organisms.

*Part A.*—One platinum loopful of 24 hours' old broth culture of *B. enteritidis* (Gaertner D.) added to each of three malachite green tubes. One platinum loopful of a similar broth culture of *B. coli* (+ d) added to the same malachite green tubes.

The tubes were incubated at 37° C. for 22 hours, and then one loopful of each fluid was distributed over several plates containing F.A. media.

The results showed that no growth had taken place in the 0.1 malachite green tube; that *B. coli* had survived, but in markedly diminished numbers, in the 0.05 malachite green tube, while *B. enteritidis* had not grown at all; and in the 0.025 malachite green both had survived but in small numbers only.

There was no evidence of multiplication of *B. coli*.

*Part B.*—Aertryke and *B. coli* (d —) were used, and care was taken only to add minute quantities of the broth cultures used. Malachite green broth tubes of the three strengths were inoculated each with broth organisms. Immediately after the addition the number per cc. in each malachite green tube was carefully estimated by plating. It was found to be 330 Aertryke and 410 coli organisms. That is, only 3,300 Aertryke and 4,100 coli bacilli were present in the whole of the 10 cc. of malachite green dulcitate broth.

The tubes were incubated for 20 hours at 37° C. and the relative proportions re-estimated by plating.

0.1 MG.—No *B. coli* colonies on the plates. About 500 Aertryke colonies when one loopful of the fluid brushed direct.

0.05 MG.—No *B. coli* colonies on the plates, but all Aertryke.

0.025 MG.—91 per cent. of the colonies Aertryke and 9 per cent. *B. coli*.

It is also of interest to know how far actual multiplication had taken place in these different strength media. After the incubation the estimated number per cc. in the malachite broth was as follows:—

0.1 MG.—450,000 Aertryke; moderate proliferation.

0.05 MG.—About 3,000 million Aertryke; marked proliferation.

0.025 MG.—About 900 million Aertryke; marked proliferation. And 84 million *B. coli*; marked proliferation.

This experiment shows that 0.1 Malachite green broth is too strong for satisfactory work, and that 0.05 per cent. is the most suitable strength to employ.

It also illustrates the marked selective action of the malachite green. *B. enteritidis*, strain Gaertner D, as confirmed by a subsequent experiment, will not grow in 0.05 per cent. malachite broth.

*Experiment III.*—To determine whether different members of the Gaertner group will grow in 0.05 per cent. malachite green dulcete broth.

Broth cultures rather less than 24 hours' old used. One platinum loopful added to 5 cc. sterile water; mixed well, and two platinum loopfuls of the dilution added to a 0.05 per cent. malachite green dulcete broth tube.

In three instances the actual number of organisms added was computed, *i.e.*, for *B. coli* (d —) over 100,000, for Aertryke about 220, and for Schotmüller A over 100,000 added.

The inoculated tubes were incubated at 37° C. for 20 hours and then re-examined. A hanging drop preparation was made from each. The different strains tested were as follows:—

Strain used.	Hanging drop preparation (1 loopful).	Proliferation.
MacConkey C. ...	Many thousands of motile bacilli ...	++
Schotmüller A. ...	Bacilli in scanty numbers ...	+
Schotmüller B. ...	Bacilli very numerous ...	++
Paratyphoid B. ...	Bacilli fairly numerous ...	+
Gaertner D. ...	No bacilli found ...	—
Gaertner U.C.H. ...	Very numerous bacilli ...	++
B. psittacosis ...	Very numerous bacilli ...	++
Aertryke ...	Very numerous bacilli ...	++
Brion and Kayser ...	Numerous bacilli... ...	++
Coli (d +) ...	No bacilli found ...	—
Coli (d —) ...	No bacilli found ...	—

The two *B. coli* and the Gaertner D. tubes remained quite clear, the other malachite broth tubes showed marked turbidity after incubation.

In four instances the actual number of organisms present after incubation was computed. Results as follows were obtained :—

0·1 cc. Gaertner D. broth plated showed no colonies.

0·1 cc. Coli (d +) broth plated showed no colonies.

0·1 cc. Coli (d -) broth plated showed 15 colonies, or only 1,500 in the whole 10 cc. compared with over 100,000 found to be present at the start.

The Aerttryke malachite green broth, suitably diluted, showed about 99 million bacilli per cc.

This experiment shows that 7 out of the 8 strains of Gaertner group organisms could grow and multiply in 0·05 per cent. malachite green broth, but that both the *B. coli* strains did not grow.

*Experiment IV.*—To compare plain nutrient broth, dulcitate bile-salt peptone water and dulcitate malachite green (0·05 per cent.) broth as enrichment media for the Gaertner group. Paratyphoid B. and *B. coli* (d +) used. Diluted fractions of broth cultures of these two strains were added to each of the above media. The relative proportion of the organisms present was ascertained for each kind of enrichment medium, the tubes were incubated for 24 hours at 37° C. and the relative proportion of the two bacilli again estimated.

The results stated, as percentages, were as follows :—

—	<i>B. coli</i> (d +).	Paratyphoid B.
Nutrient broth. At start ... ..	74	26
Nutrient broth. After incubation ... ..	100	0
Dulcitate B.S. peptone. At start ... ..	80	20
Dulcitate B.S. peptone. After incubation ... ..	100	0
Malachite green broth. At start ... ..	84	16
Malachite green broth. After incubation ... ..	0	100

The actual estimated number of organisms present in the different enrichment media per cubic centimetre at the start of the experiment were respectively :—In nutrient broth 710 coli and 250 paratyphoid, in dulcitate B.S. peptone 740 coli and 185 paratyphoid, and in the malachite green 525 coli and 100 paratyphoid.

The paratyphoid bacilli had only proliferated moderately in the malachite green broth, *i.e.*, about 60 fold. Incubated for a second 24 hours they showed, however, much greater growth. In this experiment the malachite green broth showed itself a perfect enrichment medium, while the other two showed precisely opposite results.

*Experiment V.*—Similar to Experiment IV., but using a different Gaertner strain.

MacConkey C. and *B. coli* (d +) used.

The details of the experiment are exactly the same as for Experiment IV., except that the incubation at 37° C. was for 20 hours.

The results, stated as percentages, were as follows :—

—	B. coli (d +).	MacConkey C.
Nutrient broth. At start ... ..	90	10
Nutrient broth. After incubation ... ..	94·5	5·5
Dulcete B.S. peptone. At start ... ..	92	8
Dulcete B.S. peptone. After incubation ... ..	30	70
Malachite green broth. At start ... ..	93	7
Malachite green broth. After incubation ... ..	0	100

Only quite small numbers were added as in the last experiment.

Extensive proliferation of MacConkey C. had taken place in all the enrichment media, but most abundantly in the malachite green broth.

In this experiment also the malachite green broth yielded the best results.

*Experiment VI.*—To determine the value of dulcete malachite green broth to isolate Gaertner organisms from faeces.

An emulsion of quite fresh human excreta in 15 cc. of sterile water prepared. A diluted fraction of a 24 hours' old broth culture of paratyphoid B. added to the emulsion.

The total number of paratyphoid bacilli added was carefully estimated, and was found to be about 4,110 bacilli in the whole 15 cc. The number of B. coli was also estimated, and equalled about 32,000 B. coli in each cc. of the emulsion.

That is, each cc. of the emulsion contained about 32,000 B. coli and 270 paratyphoid bacilli.

1 cc. of the excreta emulsion was added to a tube of 0·05 malachite green broth=dilution  $\alpha$ .

1 cc. of dilution  $\alpha$ , after thorough mixing, was added to another tube of malachite broth=dilution  $\beta$ .

Each malachite green broth tube contained exactly 10 cc. of medium.

According to the above computation dilution  $\beta$  should contain about 3,000 B. coli and 25 paratyphoid bacilli in the whole 11 cc., thus approximating to conditions which might be met with in excreta. Both tubes were incubated at 37° C. for 20 hours, and the relative abundance of the two organisms again determined by brushing diluted fractions over suitable media.

The plates showed that *B. paratyphosus* had multiplied abundantly in both the tubes, but that no *B. coli* colonies were present on any of the plates.

In this experiment, therefore, the naturally present *B. coli* of human excreta were quite suppressed, while the paratyphoid bacilli readily multiplied, and this although at the start the coli bacilli were 120 times as numerous as the paratyphoid bacilli.

*Experiment VII.*—Similar to Experiment VI., but using another strain of the Gaertner group and a fresh excreta emulsion.

An emulsion of quite fresh human excreta in 30 cc. of sterile water prepared to which was added a diluted fraction of a 24 hours' old broth culture of Aerttryke bacillus.

Careful platings showed that there were about 55 *B. Aertryke* and 5,330 *B. coli* in each cc. of the excreta emulsion.

1 cc. of the emulsion was added to a tube of malachite green broth=dilution  $\alpha$ .

1 cc. of dilution  $\alpha$  was added to a tube of malachite green broth=dilution  $\beta$ .

According to the above computation dilution  $\beta$  should contain about 5 *Aertryke* bacilli and 485 *B. coli* in the whole 11 cc. Both tubes were incubated at 37° C. for 20 hours, and the relative abundance of the two organisms was again determined by brushing diluted fractions over suitable media.

*Dilution  $\alpha$ .*—The plates made from this dilution showed innumerable *Aertryke* colonies, but no *B. coli* colonies, showing that marked proliferation of the *Aertryke* bacilli had taken place in the malachite green broth, but that *B. coli* had been unable to multiply.

*Dilution  $\beta$ .*—The plates showed no *Aertryke* or *coli* colonies and the broth remained clear.

A few *Aertryke* organisms were certainly added to this malachite green broth tube because, apart from the above computation, two separate 0.1 cc. fractions of dilution  $\beta$  were each respectively brushed, before incubation, over a F.A. plate. On the one plate 9 *B. coli* only developed and on the other 7 *B. coli* and one *Aertryke*. When only an excessively few Gaertner organisms are present they may therefore not be able to multiply.

The results of Experiments VI. and VII. are very striking, showing as they do the marked inhibitory power of malachite green upon the *B. coli* of excreta, while no inhibition was exerted upon the two Gaertner group organisms experimented with, but on the contrary they proliferated freely.

*Experiment VIII.*—A continuation of Experiment VII.

The 30 cc. excreta emulsion, containing *Aertryke* bacilli, of Experiment VII., was allowed to stand at room temperatures for 24 hours. Then 1 cc. was added to a malachite green broth tube. This was incubated at 37° C. for 20 hours and then fractions of it plated. No *B. coli* colonies developed but only *Aertryke* colonies.

This experiment is introduced to show that the artificial condition outside the body for 24 hours did not enable the *B. coli* to grow in the malachite green broth any better than before, while it was still possible to readily isolate the *Aertryke* organisms.

*Experiment IX.*—To test whether certain recently isolated organisms of the Gaertner group will grow in 0.05 per cent. malachite green dulcete broth.

MG<sub>2</sub>, MG<sub>6</sub>, the sources which have already been described and G<sub>4</sub>, G<sub>7</sub>, and G<sub>8</sub>, derived from animal excreta (*vide* Table II. *infra*) were used.

Diluted fractions of 20 hours' old broth cultures of these organisms were each inoculated into a tube of malachite green broth. After 20 hours' incubation at 37° C. the tubes were again examined.

The tubes inoculated with MG<sub>2</sub> and MG<sub>6</sub> were very turbid, and showed abundant growth.

The tubes inoculated with the other three organisms were quite clear, and showed no signs of growth.

The three latter tubes were then re-inoculated but with one loopful of the broth culture direct. They showed no growth next day, but after three days' incubation  $G_4$  and  $G_7$  showed well marked growth, while  $G_8$  remained sterile.

*Experiment X.*—On the same lines as Experiment IX. but with different organisms.

The bacteria used were  $MG_9$  (Table I.), and  $G_1, G_3, G_9, G_{10}, G_{11}, G_{12}$  (Table II.). Approximately equal diluted fractions of 20 hours' old broth cultures of these organisms were each inoculated into a tube of 0.05 per cent. malachite green dulcitate broth. The number of organisms added was estimated in three instances. For  $G_1$  and  $G_3$  it was about 200, for  $MG_9$  less than 100 bacteria.

After 24 hours' incubation at 37°C. the tubes were again examined.

$MG_9$  broth was very turbid, and showed abundant growth.

All the others, *i.e.*, the  $G_1, G_3, G_9, G_{10}, G_{11}, G_{12}$ , inoculated tubes were perfectly clear. These six tubes were then re-inoculated each with a loopful of broth culture. All six tubes on re-incubation showed no growth.

The results of these two experiments are of peculiar interest, since the only three organisms which grew readily in the malachite green broth were  $MG_3, MG_6$ , and  $MG_9$ , undoubtedly Gaertner organisms which readily react with Gaertner sera.

On the other hand, although  $G_7, G_8$ , and  $G_{12}$  culturally were members of the Gaertner group,\* yet none of them reacted with the sera of animals immunised with known members of the Gaertner group. The other organisms which would not grow in M. G. broth were still further removed from, although closely allied to, the typical group organisms.

In other words, simple inoculation into malachite green broth had sorted out these organisms sharply into those which reacted to Gaertner sera and those which did not, and this irrespective of whether their cultural characters were quite identical with the common Gaertner type or not.

The two experiments indicate that the malachite green broth may not only be of use for isolation but may be of great value as a group test, serving to differentiate the true members of the group.

These experiments, as a whole, show that malachite green dulcitate broth acts as a true enrichment medium in that it allows the required organisms to multiply while it inhibits the growth of those likely to obscure and confuse.

The results also show that there is a possibility that the inhibitory action may be exerted, not only against *B. coli*, but also upon certain strains which belong to or are closely allied to the Gaertner group. Otherwise its use as an enrichment medium was completely satisfactory.

Considering the whole question of suitable methods available for the isolation of members of the Gaertner group from animal excreta, or intestinal contents, the following conclusions can be drawn:—

- (1.) Animal inoculation methods are not trustworthy.

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\* See, however, footnote on page 277.

- (2.) Fuchsin agar and lactose bile-salt neutral-red agar are media which allow Gaertner group organisms to be sharply differentiated from *B. coli* organisms, and are of great value in the isolation of members of this group.
- (3.) Malachite green in the strength of 0.05 per cent. exerts a powerful inhibitory action upon the growth of *B. coli*.  
Very successful results were obtained in the isolation of Gaertner organisms when a dulcitate malachite green broth was employed containing 0.05 per cent. of malachite green.
- (4.) There is a possibility that not all the members of the Gaertner group will flourish in 0.05 per cent. malachite green broth, and that in view of this, direct plating methods should also be employed, at any rate provisionally.
- (5.) The best method for the isolation of members of this group is a combination of direct plating of the material, adequately diluted, upon suitable media such as fuchsin agar and lactose bile-salt neutral-red agar and incubation of some of the material in 0.05 per cent. malachite green dulcitate broth for 20 to 24 hours with subsequent distribution over plates of the same solid media.

## PART II.

### *A report upon the results of an examination of the intestinal contents of certain presumably healthy animals.*

The examinations included in this report were all made from materials taken from presumably healthy animals, since it is desirable in the first place to ascertain how far members of the Gaertner group are to be found in the normal gut and excreta.

Although from the point of view of potential harmfulness it is of greater importance to ascertain if these organisms are present in the excreta rather than in the intestinal canal itself, yet if they are detected in the latter it may be presumed that they will be voided in the excreta.

For the dysentery group of bacteria it has been found that they are more readily detected in the mucous membrane scrapings than in the actual mass of the contents, and it may be accepted as a reasonable assumption that if members of the Gaertner group are present in the gut, they are more likely to be found in the mucous membrane scrapings than in the actual contents.

For all the animals examined primary attention was therefore given to the examination of scrapings of the mucous membrane. In every case the material examined was quite fresh. The animals were slaughtered and the pieces of gut were removed *immediately* afterwards. This also enabled an examination of the animal to be made.

In every case the examination of the intestinal contents was commenced within two hours of death, and usually within one hour. In no cases were any abnormalities or diseased conditions

noticed in the slaughtered animals, and they were all used for food.

The primary object being to ascertain if organisms of this group were ever present and negative results being probable, it is of greater importance to investigate the cases examined fully rather than to deal with a larger number but in a superficial manner since in the latter case negative results can have but little value.

For each of the animals investigated the examination was therefore reasonably searching. In each instance three separate pieces of gut were examined, *i.e.*, a piece of small intestine, a piece of large intestine (colon), and part of the cæcum.

From 6 to 9 inches of intestine was ligatured on each side, and the ligatured pieces removed to the laboratory with their contents untouched.

In order that the value of any negative results obtained may be appraised the actual number of plates, &c. used are given. The present part of the report deals with the examination of six pigs, three bullocks, and one calf.

They are given seriatim and in the order in which they were made.

### *Intestinal Examinations.*

*No. 1.* Fig.—The procedure is given more in detail for this case, subsequent examinations being very similar.

The ligatured pieces of gut were cut open with a sterile knife and the contents evacuated as far as possible. In some instances sterile water was employed for this purpose. The mucous membrane was then carefully scraped with a sterile knife and the scrapings added to 15 cc. of sterile water. The same procedure was adopted for each separate piece of gut. Usually the emulsion also contained a small quantity of the intestinal contents itself.

Small quantities of the mucous membrane scrapings were distributed direct over solid media (L.B.A. and F.A.), also definite quantities of the emulsion. With a little experience it was found easy to obtain quite discrete and not too numerous colonies on the different plates (except the first one used, when the mucous membrane was directly brushed).

The number of organisms in the small intestine was always much less than in the other intestinal pieces, so that proportionately a larger amount of material had to be used. Plates made in this way directly from the material, without preliminary incubation, are spoken of in this report as *primary plates*.

In addition enrichment media were used.

In this particular examination 1 cc. of each emulsion was added to a tube of 0.05 malachite green dulcete broth (MG broth for brevity), and 0.2 cc. of the emulsion for the large intestine and cæcum, and 1 cc. for the small intestine each to a tube of dulcete bile-salt peptone water. The six tubes were then incubated at 37° C. for 20 hours, and suitable fractions of the liquids distributed over L.B.A. and F.A. plates. Plates made from these enrichment fluids after incubation are called in this report *secondary plates*.

The actual number of plates inoculated in this examination were as follows :—

Large intestine	{ 7 primary plates. 8 secondary plates.
Small intestine	{ 8 primary plates. 7 secondary plates.
Cæcum	{ 7 primary plates. 4 secondary plates.

In all, therefore, 41 plates were brushed, while 43 colonies were subcultivated and investigated.

The plates used were for the most part large, with a diameter of  $4\frac{1}{2}$  inches for the lower dish.

The great majority of the white colonies examined were non-lactose fermenters of the following type :—Glucose fermented with acid and gas production, lactose and dulcitate not fermented, milk acid production with coagulation of the milk after a few days' incubation.

No typical Gaertner group organisms were isolated.

The characters of the organisms isolated are more conveniently dealt with altogether, and are discussed subsequently.

The procedure found most convenient in dealing with the investigated organisms was to subcultivate direct into lactose peptone water and incubate for two or three days at 37° C. All the tubes which showed no lactose fermentation were then inoculated into litmus milk and dulcitate peptone water. These were further subcultivated if found to be of interest.

No. 2. Bullock.—Procedure as for No. 1, except that the enrichment media were differently inoculated.

Dulcitate bile-salt peptone water, 0.05 MG broth and 0.025 per cent. MG were all used, the tubes being directly inoculated with mucous membrane scrapings.

The plates inoculated were as follows :—

Large intestine	{ 6 primary plates. 4 secondary plates.
Small intestine	{ 6 primary plates. 4 secondary plates.
Cæcum	{ 6 primary plates. 6 secondary plates.

In all 32 plates were brushed and 48 colonies subcultivated and investigated.

The plates were rather thickly covered with colonies, although these were mostly discrete, and the majority of those subcultivated were lactose fermenters. Extremely few non-lactose fermenters were met with, and no organisms which could possibly belong to the Gaertner group. Indeed, the only organism of any interest was a member of the *B. fecalis alkaligenes* group.

No. 3. Pig.—Procedure as for No. 1, except that the enrichment media were differently inoculated.

For each piece 1 cc. of emulsion was added to a tube of 0.05 per cent. MG broth = dilution  $\alpha$ .

1 cc. of dilution  $\alpha$  after mixing added to a second tube of MG broth = dilution  $\beta$ . Secondary plates made after 20 hours' incubation at 37° C.

The plates inoculated were as follows :—

Large intestine	{ 4 primary plates. 8 secondary plates.
Small intestine	{ 4 primary plates. 5 secondary plates.
Cæcum	{ 6 primary plates. 7 secondary plates.

In all 34 plates were brushed and 51 colonies subcultivated and investigated.

The white colonies were not very numerous, and it was possible to subcultivate the majority of them.

Only one organism, G<sub>3</sub>, Table II., was isolated, which fermented glucose but not lactose, and turned milk alkaline after preliminary acid production. This was obtained from the secondary plates of the cæcum. Other interesting non-lactose fermenters were isolated and are considered later.

No. 4. Bullock.—Procedure exactly the same as for No. 3.

The plates inoculated were as follows :—

Large intestine	{ 7 primary plates. 3 secondary plates.
Small intestine	{ 6 primary plates. 5 secondary plates.
Cæcum	{ 6 primary plates. 3 secondary plates.

In all 30 plates were brushed. Practically no white colonies were met with, and only four suspicious colonies were subcultivated. Two of these fermented lactose, while the other two neither fermented glucose nor lactose.

No Gaertner group organisms were therefore met with.

No. 5. Pig.—Procedure quite similar to the last two cases, except that for the small intestine no dilution  $\beta$  was made, but a small quantity of the mucous membrane was added direct to a tube of MG broth = dilution  $\alpha\alpha$ .

The plates inoculated were as follows :—

Large intestine	{ 7 primary plates. 3 secondary plates.
Small intestine	{ 6 primary plates. 4 secondary plates.
Cæcum	{ 7 primary plates. 3 secondary plates.

In all 30 plates were brushed, and 58 colonies subcultivated and investigated.

Most of the organisms isolated slowly fermented lactose. They appeared on the plates as white colonies.

Three organisms of the Gaertner group were isolated.

No. 6. Bullock.—Procedure exactly as for No. 5, except that in addition tubes of dulcitate neutral-red bile-salt peptone water were inoculated with 1 cc. of the emulsion from each piece of gut.

The plates inoculated were as follows :—

Large intestine	{ 8 primary plates. 4 secondary plates.
Small intestine	{ 6 primary plates. 4 secondary plates.
Cæcum	{ 7 primary plates. 6 secondary plates.

In all 35 plates were brushed. Extremely few white colonies were present, and it was only necessary to subcultivate and investigate 17 colonies. The majority of these fermented lactose and no Gaertner group organisms were met with, the only organism of any interest being a member of the *B. faecalis alkaligenes* group.

No. 7. Fig.—Procedure as for No. 6, except that after the dulcitate bile-salt peptone water tubes had been incubated for 20 hours, 1 cc. of the fluid was added to a fresh malachite green broth tube (dilution α). This was incubated until next day and then plated. Such plates are here called *tertiary plates*.

The plates inoculated were as follows :—

Large intestine	{ 8 primary plates. 6 secondary plates. 1 tertiary plate.
Small intestine	{ 5 primary plates. 5 secondary plates. 2 tertiary plates.
Cæcum	{ 8 primary plates. 6 secondary plates. 1 tertiary plate.

In all 42 plates were brushed, and 42 colonies subcultivated and investigated.

Only one of these organisms could in any way be considered a member of the Gaertner group. The majority was non-lactose fermenters which coagulated milk after a good many days incubation.

No. 8. Fig.—Procedure similar to the last, except that the enrichment tubes were slightly different.

1 cc. of the emulsion of each specimen was added to a tube of MG broth and 1 cc. to a dulcitate bile-salt peptone water tube. These incubated and secondary plates made in the usual way. Also four platinum loopfuls of each dulcitate bile-salt tube, after 20 hours' incubation, were added to fresh MG broth tubes. The latter were incubated for a further 20–24 hours. In all three cases although numerous organisms had been added no growth resulted.

The plates inoculated were as follows :—

Large intestine	{ 7 primary plates. 5 secondary plates.
Small intestine	{ 5 primary plates. 6 secondary plates.
Cæcum	{ 7 primary plates. 5 secondary plates.

In all 35 plates we brushed, and 40 colonies subcultivated and investigated.

A good many white colonies were present, but typical Gaertner group organisms were not met with.

No. 9. Fig.—Procedure exactly similar to No. 8.

The malachite green tubes inoculated from the dulcete peptone water here showed well-marked growth after 24 hours, but when plated showed only red colonies.

The plates inoculated were as follows :—

Large intestine	{	8 primary plates.
	{	4 secondary plates.
	{	2 tertiary plates.
Small intestine	{	5 primary plates.
	{	4 secondary plates.
	{	2 tertiary plates.
Cæcum	{	7 primary plates.
	{	4 secondary plates.
	{	2 tertiary plates.

In all 38 plates were brushed, and 75 colonies subcultivated and investigated.

White colonies were fairly numerous, and were mostly of one or other of two groups. Both groups fermented glucose and dulcete but not lactose, and produced acid in milk. In the one group the milk remained permanently acid without coagulation, in the other coagulation took place, but very slowly, and usually only after two weeks or more incubation. No typical Gaertner group organisms were met with.

*No. 10.* Calf about two months old.—Procedure very similar. Both dulcete bile-salt peptone water and malachite green broth were used as enrichment media.

For the small intestine inoculations small amounts of mucous membrane scrapings were added direct to these two tubes, but for the large intestine and cæcum one cc. of the emulsion of the scrapings was added in the usual way.

The plates inoculated were as follows :—

Large intestine	{	7 primary plates.
	{	4 secondary plates.
Small intestine	{	5 primary plates.
	{	2 secondary plates.
Cæcum	{	7 primary plates.
	{	2 secondary plates.

The MG broth tubes from the small intestine and cæcum showed no growth.

In all 27 plates were brushed, and 32 colonies subcultivated and investigated.

Only lactose fermenters were obtained from the small intestine plates, but white colonies were very numerous on the plates from the two other pieces of intestine. All those investigated were quite identical and were equally numerous in both the large intestine and the cæcum. In their cultural characters they were typical Gaertner group organisms. To illustrate their actual abundance the following results obtained may be quoted :—

Large intestine: primary plates; one loopful of emulsion distributed over one F.A. plate. Next day, 112 red colonies, 23 white. Three of the latter culturally worked out proved to be identical and typical Gaertner organisms.

$\frac{1}{30}$  cc. of same emulsion distributed over three plates. Next day, first plate (L.B.A.) overcrowded but colonies mostly discrete, a good many white colonies. Second plate (F.A.) 120 discrete red colonies and 10 white. Third plate (L.B.A.) 14 red colonies only. Four of the white colonies were worked out. The organisms were identical with the above. Cæcum-primary plates. One loopful of emulsion distributed over one F.A. plate. Next day 92 red and 6 white colonies. Three of them subcultivated and worked out.

$\frac{1}{10}$  cc. of same emulsion brushed over three plates. First plate overcrowded. Second plate (L.B.A.) 172 red and 30 white colonies. Third plate (F.A.) 14 red and 2 white colonies. Two of the colonies culturally investigated. All five organisms identical with the above.

These figures show that these Gaertner organisms were quite abundant.

The bacteria obtained from the 10 animals can be conveniently considered together. Those obtained from the three bullocks can be briefly dealt with. Extremely few white colonies of any kind were met with, and most of these within one or more days fermented lactose when subcultured into that medium.

No organisms which in any way could be considered members of the Gaertner group were isolated. In two instances *B. faecalis alkaligenes* was found, but otherwise practically all the colonies which developed upon the L.B.A. and F.A. plates were ordinary lactose fermenting *B. coli*.

The intestinal contents and mucous membranes of the six pigs and the calf showed on the other hand white colonies which were as a rule numerous.

These organisms can be classified into three classes:—

- (A.) Those which ferment lactose slowly.
- (B.) Those which do not ferment lactose, but produce acid and clot in milk, the coagulation of the milk not as a rule resulting until after one or more weeks' incubation.
- (C.) Organisms which ferment glucose but not lactose, and which do not coagulate milk.

Class (C) organisms are the only bacteria of any interest in the present report and which need be further considered.

These organisms readily fall into two groups, each of which can be again divided. The four groups in this way obtained are:—

- (1.) Bacteria which produce permanent acidity in milk; dulcitate not fermented.
- (2.) Bacteria which produce permanent acidity in milk; dulcitate fermented.
- (3.) Bacteria which produce an alkaline reaction in milk; dulcitate not fermented.
- (4.) Bacteria which produce an alkaline reaction in milk; dulcitate fermented.

Group (1) organisms were met with in two animals, *i.e.*, pigs Nos. I. and V.  $G_2$  and  $G_5$ , Table II., show the cultural characters of such organisms.

TABLE II.

Designation of organism.	Source.	Glucose fermentation.	Lactose fermentation.	Dulcitol fermentation.	Saccharose fermentation.	Neutral-red reaction.	Litmus milk.	Liquefaction of gelatine.	Indol production.	Motility.	Pathogenicity.
G <sub>1</sub>	I. (Pig) large intestine	+	-	+	-	+p	Acid no clot	-	+	+	-
G <sub>2</sub>	I. (Pig) large intestine	+	-	-	-	+p	Acid no clot	-	+	+	
G <sub>3</sub>	III. (Pig) caecum ..	+	-	-	-	+	Acid then alkaline.	-	-	+	!
G <sub>4</sub>	III. (Pig) all three parts	+	-	+	-	+	Acid no clot	-	+	+	+
G <sub>5</sub>	V. (Pig) small intestine	+	-	-	-	+	Acid no clot	-	-	+	
G <sub>6</sub>	V. (Pig) large intestine	+	-	+	+	+	Acid then alkaline.	-	+	+	
G <sub>7</sub>	V. (Pig) large intestine	+	-	+	+	+	Acid then alkaline.	-	+	+	+
G <sub>8</sub>	V. (Pig) caecum ..	+	-	+	+	+	Acid then alkaline.	-	+	+	+
G <sub>9</sub>	VII. (Pig) small intestine	+	-	+	-	+	Acid no clot	-	+	+	
G <sub>10</sub>	VIII. (Pig) large intestine	+	-	+	-	+	Acid no clot	-	+	+	
G <sub>11</sub>	IX. (Pig) all three parts	+	-	+	-	+	Acid no clot	-	+	+	-
G <sub>12</sub>	X. (Calf) caecum and large intestine.	+	-	+	-	+	Acid then alkaline.	-	-	+	+

+p = partial reaction.

Group (2) organisms were met with in five intestinal contents, all from pigs, *i.e.*, Nos. I., III., VII., VIII., IX. In only one pig intestine were they not found. As a rule they were fairly numerous and easily found.

G<sub>1</sub>, G<sub>4</sub>, G<sub>9</sub>, G<sub>10</sub>, G<sub>11</sub>, Table II., are examples of such organisms. As this table shows they are all identical.

Group (3) organisms were met with in only one animal, No. III., a pig, and only a single organism of this group was isolated. Its cultural characters (G<sub>3</sub>) are given in Table II.

Group (4) organisms were only met with in two animals, *i.e.*, Nos. V. and X., a pig and a calf.

Only three bacteria of this group were obtained from the pig, *i.e.*, G<sub>6</sub>, G<sub>7</sub>, G<sub>8</sub>, but in the calf they were very numerous and all identical, their characters corresponding with G<sub>12</sub>, Table II.

The different members of the Gaertner group culturally fall into two divisions according as to whether they produce alkalinity in milk, after preliminary acid production, or produce permanent acidity.

The first division includes all the known Gaertner members with the exception of the paratyphoid *A.* sub-group—these constitute the other group.

How far the power to ferment dulcitol is to be considered a definite and always present characteristic of the Gaertner group is at present undecided.

Certainly almost all the known pathogenic varieties ferment this substance, but with our present knowledge it is unjustifiable

to assume that organisms which do not ferment dulcete are therefore not Gaertner group organisms.

Although the members of the first division are culturally, for the most part, identical they can be further differentiated by their agglutinative properties.

Three sub-groups can be fairly definitely recognized. They are :—

- (a.) The hog-cholera sub-group, including amongst others the bacilli at one time etiologically associated with hog-cholera and some of the bacilli derived from outbreaks of food-poisoning (*B. Aertryke*, &c.).
- (b.) The Gaertner sub-group, including *B. enteritidis* and some other meat poisoning bacilli.
- (c.) Paratyphoid B sub-group, including *B. paratyphosus* B.

The organism *B. psittacosis*, isolated from a disease of parrots, may also be a member of a separate sub-group.

These sub-groups with (d) the paratyphoid A sub-group, which includes the organisms Brion and Kayser and Schotmüller A form the chief (at present) recognized sub-groups of the Gaertner group, the distinctions between the groups being, except for sub-group (d), decided by agglutinative affinities alone.

Groups (1) and (2) described above may be included in the paratyphoid A sub-group, while groups (3) and (4) are culturally allied to the Gaertner organisms which produce alkalinity in milk.

The three organisms of group (4) derived from the pig are, on closer investigation, distinguished from the known members of the Gaertner group by the fact that all three of them ferment saccharose. On the other hand, all the organisms obtained from the calf, identical with G<sub>1</sub>, failed to ferment saccharose.

For the further determination of the affinities of these bacteria recourse must be made to agglutination tests.

Four rabbits were immunized against four known varieties of Gaertner organisms, i.e., paratyphoid B, *B. enteritidis* (Gaertner D), Schotmüller A, and Aertryke.

They were immunized by injections first of killed cultures, later of living cultures.

The Schotmüller A rabbit, even after repeated injections developed but little agglutination power. A fresh rabbit was, therefore, immunized with the Brion and Kayser strain, while a sixth rabbit was immunized against G<sub>7</sub>.

Only sera of a moderate agglutination power were obtained. The Gaertner, Aertryke, and paratyphoid injected rabbits showed a positive reaction of 1 : 5,000, while the Brion and Kayser rabbit showed a positive reaction both when diluted 1 : 5,000 and 1 : 10,000, but in both cases the reaction was rather incomplete.

The G<sub>7</sub> rabbit gave a marked reaction 1 : 1,000, but no reaction 1 : 10,000.

The Schotmüller A rabbit gave only an incomplete positive reaction in a 1 : 1,000 dilution.

The agglutination reactions of the different organisms of Table II, are shown in Table III. :—

TABLE III.

Bacillus.	Paratyphoid B.		Gaertner.		Aertryke.		Brion and Kayser.		Schotmüller A.		G, Serum.	
	1 in 50.	1 in 100.	1 in 50.	1 in 100.	1 in 50.	1 in 100.	1 in 50.	1 in 100.	1 in 50.	1 in 100.	1 in 50.	1 in 100.
G 1	—	—	—	—	—	—	—	—	—	—	—	—
G 3	—	—	—	—	—	—	—	—	—	—	—	—
G 4	—	—	—	—	—	—	—	—	—	—	—	—
G 6	—	—	—	—	—	—	—	—	—	—	—	—
G 7	—	—	—	—	—	—	—	—	—	—	—	+
G 8	—	—	—	—	—	—	—	—	—	—	—	+
G 9	—	—	—	—	—	—	—	—	—	—	—	+
G10	—	—	—	—	—	—	—	—	—	—	—	—
G11	—	—	—	—	—	—	—	—	—	—	—	—
G12A	—	—	—	—	—	—	—	—	—	—	—	—
G12B	—	—	—	—	—	—	—	—	—	—	—	—
G12C	—	—	—	—	—	—	—	—	—	—	—	—

All the tests were microscopic, in hanging drop preparations. For the 1 in 50 dilution, time allowance one hour. For the 1 in 100 and higher dilutions, two hours allowed. Young broth cultures used throughout, incubated for 18 to 24 hours, either at 37° C. or 21° C., or partly both. + = well-marked distinct clumps with or without some bacilli between. — = a negative reaction, no clumps being present. G12A, G12B, G12C are three separate organisms from the calf intestine, all culturally identical with one another.

Table III. shows that none of the organisms isolated agglutinate, even in dilutions of 1 in 50 and 1 in 100, sera obtained from typical members of the Gaertner group. These sera were selected so as to include all the chief types of Gaertner organisms, and failure to agglutinate in these dilutions is strong evidence that these organisms are not members of these groups.

This was partially indicated by the cultural characters of G<sub>6</sub>, G<sub>7</sub>, G<sub>8</sub>, in that they ferment saccharose.

G<sub>12</sub>, however, was culturally undistinguishable from the main division of Gaertner organisms.\*

The reactions of G<sub>6</sub>, G<sub>7</sub>, and G<sub>8</sub> with G<sub>7</sub> serum show that the three organisms are closely allied if not identical. All three show precisely similar results, giving a well-marked reaction in dilutions of 1 in 1,000, but active motility with no trace of agglutination in a dilution of 1 in 10,000.

G<sub>12</sub> is not agglutinated by this serum, even in a dilution of 1 in 50.

Table III. shows that the organisms isolated do not belong to any of the known types of the Gaertner group. Their significance, if any, is at present undetermined, and a question for further investigation.

The agglutination reactions of certain of the bacteria described in Table I. are of interest.

These are described in Table IV. :—

\* By the use of additional cultural tests, particularly the fermentation of *salicin*, it has been found possible to culturally separate these organisms from the recognised members of the Gaertner group. These tests will be detailed in a subsequent report.

TABLE IV.

Serum and dilution.				MG <sub>3</sub>	MG <sub>4</sub>	MG <sub>5</sub>
Paratyphoid B.	...	...	1 in 100	+	+	+
Paratyphoid B.	...	...	1 in 1,000	—	—	+
Paratyphoid B.	...	...	1 in 5,000	—	—	+
Paratyphoid B.	...	...	1 in 10,000	—	—	+
Gaertner	...	...	1 in 100	—	—	—
Aertryke	...	...	1 in 100	+	+	+
Aertryke	...	...	1 in 1,000	+	+	+
Aertryke	...	...	1 in 5,000	—	+	incomplete.
Aertryke	...	...	1 in 10,000	—	Partial	—
Brion and Kayser	...	...	1 in 100	+	+	+
Brion and Kayser	...	...	1 in 500	—	—	—
Schotmüller A.	...	...	1 in 100	—	—	—
G, Serum	...	...	...	—	—	—

Table IV. shows that MG<sub>3</sub> and MG<sub>4</sub> can from their agglutination properties be assigned to the Aertryke or hog-cholera sub-group, while MG<sub>5</sub> belongs to the paratyphoid B sub-group.

A point of interest in this inquiry is that no organisms of the *B. dysenteriae* group were met with. These organisms grow upon F.A. and L.B.A. media, and the possibility of their presence was kept in mind. No organisms of this kind were met with.

The results of this part of the present inquiry may be briefly summarised as follows:—

- (1.) In none of the three bullocks examined were organisms remotely resembling Gaertner group organisms met with.
- (2.) In the six pigs examined non-lactose fermenters were fairly numerous. Organisms culturally resembling the paratyphoid A sub-group were isolated from five pigs, and when present were moderately numerous.

Only four organisms were isolated which were culturally allied to the other and more distinctive Gaertner sub-groups, while these on closer examination could be distinguished culturally, since the one did not ferment dulcitol while the other three fermented saccharose, a character not possessed by the known members of this group.

- (3.) All the organisms isolated from the pigs failed to be agglutinated by sera obtained from rabbits immunized from well-known members of the group.
- (4.) The only calf examined showed both in its large intestine (colon) and in the cæcum numerous organisms which culturally were undistinguishable from the ordinary Gaertner group bacteria; and which possessed a high degree of pathogenicity.

When tested against the different immune sera these organisms failed to react with any one of them, and must be provisionally assumed to belong to a separate sub-group.

How far they are common in the intestines of calves, and to what extent their presence constitutes a menace to the health of those using such animals is a question which requires further elucidation.

## APPENDIX B, No. 6.

REPORT on a STUDY of the MICRO-ORGANISMS ASSOCIATED with RHEUMATIC FEVER and MALIGNANT ENDOCARDITIS, by T. J. HORDER, M.D., B.Sc., F.R.C.P.

## SCHEME OF REPORT.

## SECTION I.—INTRODUCTORY.

Present ignorance of the essential cause of rheumatic fever. The microbic theory. The search for micro-organisms in affected persons. The discordant nature of the results. The great frequency of negative results. The constant association of micro-organisms with malignant endocarditis. The nature of the micro-organisms isolated. The need for observations conducted *in vivo*.

## SECTION II.—CLINICAL AND BACTERIOLOGICAL.

Blood cultures. The technique employed. The results in thirty-two cases of rheumatic fever examined *in vivo*. The results in thirteen cases examined *post mortem*. An analysis of thirty cases of positive blood cultures in cases of malignant endocarditis. The characters of the micro-organisms obtained. The streptococcal group. The streptococci in malignant endocarditis do not conform to the type *S. pyogenes* but to *S. salivarius* and *S. faecalis*.

## SECTION III.—EXPERIMENTAL INVESTIGATION.

The production of endocarditis by the injection into rabbits of *S. salivarius* isolated from a case of malignant endocarditis. The production of endocarditis by the injection of streptococci obtained from normal human saliva and normal human faeces. Observations upon animals so injected. Comparison of these animals and animals injected with highly "pathogenic" micro-organisms (*pneumococcus* and *S. pyogenes*).

## SECTION IV.—GENERAL CONCLUSIONS.

Summary of results. The bearing of these results upon the present theories of the causation of rheumatic fever.

## SECTION I.

## INTRODUCTORY.

The problem of the essential cause of *rheumatic fever* is one that still awaits solution despite much attention on the part of clinicians, pathological chemists, and bacteriologists. A knowledge of the true *materies morbi* concerned in this disease would constitute one of the greatest possible boons to humanity in general, and to this country in particular. For the malady is one of the commonest amongst those who, by virtue of their age and occupation, have just entered upon active physical life. The frequent tendency to recurrences with long periods of tardy convalescence, and especially the permanent crippling of the heart, too commonly seen as the direct result of the disease, cannot, unfortunately, be effectually prevented by any treatment at present known to us. These reflections amply serve to stimulate research into the cause of rheumatic fever along any lines that seem to offer hope of a successful issue.

That some poison is at work in the tissues of a patient suffering from acute rheumatism seems certain. That this poison is of a specific nature seems highly probable in face of the great constancy of the clinical picture displayed by the disease. The disease undoubtedly constitutes a definite entity. Of this there is an almost unanimity of opinion amongst clinical observers. This being so it is reasonable to expect that a specific virus exists as the chief casual factor in the disease. But whether the poison is of bacterial origin or not remains unknown.

The argument from analogy with other diseases is often made use of as supporting the theory of the bacterial origin of the rheumatic virus. But the argument is treacherous. The close resemblance often seen between rheumatic fever and such a disease as streptococcal or staphylococcal pyæmia is by some considered strong evidence in favour of rheumatic fever being due to infection by a micro-organism. But the resemblance sometimes seen between rheumatic fever and a severe attack of blood-poisoning due (say) to the subcutaneous injection of sterile horse serum is scarcely less close. The possibility, therefore, of the poison in question being a chemical substance of other than microbic manufacture must not be lost sight of.

The existence of a special seasonal incidence in the disease, and the occurrence of marked fluctuations in its prevalence at different places are facts which point more definitely towards the microbic theory of the cause of rheumatic fever (Hirsch, Newsholme, Longstaff, and others). The marked tendency to the disease manifested during the periods of youth and young adult life also supports this theory. Be this as it may, enough presumptive evidence exists to justify the investigation of cases of rheumatic fever on bacteriological lines. And such investigation has been made, in a most thorough manner, during life and after death, by various workers, with the object of isolating the hypothetical micro-organism from the infected tissues.

These investigations have so far yielded extremely discordant results. The reasons for this, as suggested by Bulloch, lie partly in the difficulty that exists in the certain diagnosis of rheumatic fever, partly in the defective technique of some investigators, and partly in interpretations being put upon some of the observed facts that are scarcely warranted. These results may be divided into positive and negative.

#### I.—Positive Results.

These fall into three groups, according as the micro-organism isolated has been (i.) an anærobic sporing bacillus, (ii.) one or other of the staphylococcus group, or (iii.) a short streptococcus or diplococcus.

(i.) *The bacillus of Achalmé.*—This micro-organism, first isolated from a case of rheumatic fever in 1891, and later in several other cases, both before and after death, by Achalmé, recurs frequently in the records of observations made by subsequent investigators. It is a Gram-positive spore-bearing bacillus, of considerable size. Thiroloix, Triboulet and Cyon, Melkich, Pic and Lesiem, and

others have described a similar micro-organism as being associated with rheumatic fever. Many careful workers, however, have failed to demonstrate it, and much of the earlier research must be discredited on account of faulty technique and insufficient care in excluding post-mortem invasions. Moreover, there are reasons for regarding this bacillus as being identical with a common micro-organism of lactic acid fermentation (Poynton and Paine) or as being identical with *B. enteritidis sporogenes* of Klein (Hewlett). Achalmé has himself more recently come to regard this bacillus as being a common saprophyte, but considers that it may develop in the blood under certain special conditions.

(ii.) *Several varieties of staphylococci* have been from time to time isolated from patients suffering from, or dead of, rheumatic fever. These have mostly been cultivated from the urine during life or from inflammatory exudates post-mortem. The fact that these cocci have not been obtained in blood cultures during life, together with the great lack of uniformity seen in the types of the staphylococci described, make it probable that the micro-organisms dealt with have been contaminations or of the nature of secondary infection. The close resemblance oft-times existing between cases of severe rheumatic fever and staphylococcal pyæmia must also be remembered in this connection.

(iii.) *A diplococcus*: "*diplococcus rheumaticus*"; "*micrococcus rheumaticus*"; "*streptococcus rheumaticus*."—During the past eight years a number of investigators have described a micro-organism, to which various names have been given, as being frequently found in cultures made before and after death in cases of rheumatic fever. Wasserman (1898) first isolated such a diplococcus from the heart's blood and endocardial vegetations of a child dead of rheumatic fever. Triboulet (1899) obtained what appears to have been a very similar coccus from another case of the disease, and was able to produce endocarditis, pericarditis, and pleurisy by injecting it into rabbits. In England, Poynton and Paine have succeeded in isolating this, or a closely allied micro-organism, from over thirty cases of rheumatic fever (1900–1906). These observers have obtained the "*micrococcus*" from the blood, joint fluid, nodules, cerebro-spinal fluid, and urine during life, and from the heart's blood, pericardium, endocardial vegetations, nodules, and brain after death. Although it is a little doubtful if, in their cases, the micro-organism isolated has been the same coccus described by Wasserman, Triboulet, and others, or if it has been the same coccus in all of their own cases, Poynton and Paine consider the identity of the micro-organism to be proved. These two observers have given the question of the bacterial origin of rheumatic fever a great deal of attention, and have expended much labour upon it. Their results have been confirmed by Beaton and Ainley Walker (in fifteen cases) and by Beattie. The description of the diplococcus originally given by Poynton and Paine has been altered in some respects in the later publications of these authors. The characters most recently assigned to it are as follows:—a Gram-positive, non-capsulated diplococcus; it grows well on gelatine and without liquefaction; it forms a slight flocculent deposit in broth with

the formation of acid; it clots milk. By intravenous injection into rabbits of large quantities of the diplococcus, Poynton and Paine were able to produce polyarthritis, bursitis, endo- and peri-carditis, pleurisy, pneumonia, nodules, chorea, and iritis. Many of these results were repeated with great constancy. Entirely similar effects followed the experimental use of their "micrococcus" in the hands of Beaton and Walker. Beattie has also obtained similar experimental results. Shaw, using micro-organisms supplied by these observers, has repeated the experiments in monkeys, and has found similar effects to be produced.

## II.—*Negative Results.*

In striking contrast with the above findings of Poynton and Paine, Beaton and Walker, and of Beattie, are the experiences of several observers who have investigated this question. Cultivations from the blood in cases of uncomplicated rheumatic fever have been reported to be consistently negative by Lenhartz, Phillips, and Menzer in Germany; by McCrae, Lewis and Longcope and Cole in America; and by Bulloch and Thompson in England. The technique of these investigators appears to have been of the most approved kind from the point of view of discovering micro-organisms in the blood-stream.

Even in bacteriological examinations made post-mortem in cases of rheumatic fever; when the material available has naturally been much more varied and more abundant than during life, negative results have been reported by Pribram, Singer, Weichselbaum, Schottmuller, and by Bulloch and Thompson. The two last-named observers report as follows concerning an unusually severe case of rheumatic fever in a boy aged fifteen years, who had had a previous attack of the disease, and had come under observation for his fatal illness with high fever, marked pericarditis and endocarditis, and many subcutaneous nodules. Of the bacteriological investigation made post-mortem in this case the authors say: "The examination of the pericardial fluid, pericardial membrane, endocardial vegetations, rheumatic nodules, yielded sterile cultures, although different media were inoculated and maintained aerobically and anaerobically. The microscopic examination of the pericardium, endocardium, and nodules failed to show any microbes."<sup>\*</sup>

In the foregoing brief analysis of results of other observers no account has been taken of cases in which there appears intrinsic evidence that the disease was not rheumatic fever. Nor has any consideration been further given to those accounts of micro-organisms which have clearly been concerned with secondary or terminal infections.

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<sup>\*</sup> See an excellent account of the pathology of acute rheumatism by Bulloch in the 2nd edition of Allbutt's *System of Medicine*. This article is accompanied by a very full set of references.

Difficult as is the problem of the exact rôle played by micro-organisms in the causation of rheumatic fever, and of the endocarditis so frequently associated therewith, it appears to be the universal opinion that in the causation of the allied condition termed *malignant endocarditis* (or ulcerative endocarditis) micro-organisms play by far the most important rôle. No doubt it is true that all transitions may be seen in the post-mortem room between the "simple" or "rheumatic" form of endocarditis and a state of endocardial infection associated with fungating outgrowths and destruction of tissue. But rheumatic fever and malignant endocarditis, both clinically and in the post-mortem room, are quite sufficiently well differentiated to allow of separation for purposes of investigation. This matter will be dealt with later (*see* page 299).

Whether the results of bacteriological research in cases of rheumatic fever be regarded as merely equivocal or as yielding a convincing preponderance of negative results, there is no doubt as to the great constancy with which all observers find it possible to isolate micro-organisms from the tissues in cases of malignant endocarditis. There is abundant evidence, moreover, that the micro-organisms so obtained constitute the essential factors in the production of the disease. The micro-organisms which have been described in this connection fall into two groups, a common group and a rare group. The micro-organisms commonly described as being present in malignant endocarditis are the streptococci, the staphylococci, the pneumococcus, and the gonococcus. The micro-organisms described as being rarely present are *B. coli*, *B. typhosus*, *B. influenzae*, *B. diphtheriae*, *B. tuberculosis*, *B. pyocyaneus*, the meningococcus, and certain streptothrices. Mixed infections are not seldom described.

Although a large number of single instances of successful blood-culture during life have been described in cases of malignant endocarditis there has appeared as yet no series of cases in which the isolation of the micro-organism has been achieved in this manner. Most of our knowledge depends upon post-mortem observations, and these, however valuable, are complicated by two difficulties: terminal infections cannot properly be excluded, and the causal micro-organism may fail to grow on culture media if death has occurred more than a few hours before the time of the autopsy. For these reasons blood-cultures which reveal the nature of the micro-organism during life, quite apart from their great value in diagnosis and treatment, are much more to be relied upon than investigations conducted after death.

#### SCOPE OF THIS REPORT.

The present investigation deals first of all with the frequency with which micro-organisms are discoverable in the blood and tissues of persons suffering from rheumatic fever and from malignant endocarditis, with special reference to the examination by means of blood-cultures during life. It then deals with the characters of the micro-organisms which have been isolated from such persons, and the relations that these micro-organisms bear to

some of those the sources of which have been investigated in previous researches conducted on behalf of the Board. These relations are extended so as to include an account of the production of endocarditis in rabbits by the injection of micrococci derived from the human alimentary canal. Lastly, the bearing which the results of the present investigation have upon the etiology of rheumatic fever and of malignant endocarditis is considered.

## SECTION II.

### CLINICAL AND BACTERIOLOGICAL INVESTIGATION.

The technique of blood-culture has been of late years so much improved that by means of this method of clinical examination the existence of micro-organisms in the circulation can be demonstrated with great constancy. The three principles underlying the method of blood-culture are: (1) that a considerable quantity of blood shall be obtained from the patient (at least 5 cc.), (2) that the blood shall be transferred to culture media without the introduction of any contamination from the skin or air and (3) that every possible encouragement shall be given for the growth of any micro-organism that may be present in the blood dealt with. The actual steps in the process used in the course of these investigations were those detailed in an article published by the author in *The Practitioner* (November, 1905).

#### A.—Blood cultures undertaken during life in thirty-two cases of rheumatic fever.

The following series of fifteen cases of rheumatic fever has been investigated during the past three years by this method of blood-culture. The patients have mostly been under observation at St. Bartholomew's Hospital or at the Great Northern Hospital. The media used for the cultures have been very variable and have included that suggested by Poynton and Paine as being specially favourable for the growth of the "*diplococcus rheumaticus*" described by them, viz.:—equal parts of broth and milk rendered slightly acid by the addition of lactic acid. In some instances a lactic-acid agar medium has been used. The cultures have been in several instances incubated under anaërobic as well as under aërobic conditions.

TABLE I.

No.	Sex.	Age.	Nature of disease.	Result.
1	Male	15	Rheumatic fever; old and recent endocarditis.	No growth.
2	"	34	Rheumatic fever; old and recent endocarditis.	"
3	—	—	Culture repeated later .. .. .	"
4	Male	24	Rheumatic fever; acute endocarditis ..	"
5	"	20	Rheumatic fever; old and recent endocarditis.	"
6	—	—	Culture repeated later .. .. .	"

TABLE I.—*continued.*

No.	Sex.	Age.	Nature of disease.	Result.
7	Male	18	Rheumatic fever; old and recent endocarditis.	No growth.
8	"	8	Chorea; acute endocarditis .. .. .	"
9	"	37	Rheumatic fever; old and recent endocarditis.	"
10	"	34	Rheumatic fever; endocarditis, pericarditis, pleurisy.	"
11	—	—	Culture repeated later .. .. .	"
12	Male	22	Rheumatic fever; old and recent endocarditis.	"
13	Female	9	Rheumatic fever; acute endocarditis ..	"
	—	—	Culture repeated later .. .. .	"
14	Male	9	Chorea; fever; endocarditis; pericarditis	"
15	Female	21	Rheumatic fever .. .. .	"

As seen in the last column of the above table these fifteen blood-cultures gave negative results in all cases. By the kindness of Dr. F. W. Andrewes, director of the Pathological Department at St. Bartholomew's Hospital, I am able to add to the above a further series of seventeen blood-cultures, undertaken by his staff, in cases of rheumatic fever and using the same technique as that adopted in the earlier series.

TABLE II.

No.	Sex.	Age.	Nature of disease.	Result.
1	Male	11	Rheumatic fever; endocarditis, pericarditis	No growth.
2	Female	21	Old (rheumatic) mitral disease; fever; acute endocarditis.	"
3	Male	14	Mitral regurgitation; fever; acute endocarditis.	"
4	"	13	Rheumatic fever; pleural effusion; endocarditis, pericarditis.	"
5	"	21	Rheumatic fever .. .. .	"
6	—	—	Culture repeated .. .. .	"
7	Male	25	Rheumatic fever; old and recent endocarditis.	"
8	Female	14	Rheumatic fever; endocarditis pericarditis, pleurisy.	"
9	"	41	Rheumatic fever .. .. .	"
10	—	—	Culture repeated .. .. .	"
11	Male	44	Rheumatic fever; pericarditis, pleurisy ..	"
12	Female	12	Rheumatic fever; pericarditis .. ..	"
13	"	39	Rheumatic fever; endocarditis .. ..	"
14	Male	10	Rheumatic fever; endocarditis, pericarditis	"
15	Female	12	Rheumatic fever .. .. .	"
16	Male	30	Rheumatic fever .. .. .	"
17	Female	10	Rheumatic fever; endocarditis .. ..	"

Here, again, there is a complete absence of positive results. These thirty-two blood-cultures, performed in cases of rheumatic fever and chorea, include the whole of the cases of this disease which have been investigated in this manner at St. Bartholomew's Hospital during the years 1905-6-7. In none of these cases has any micro-organism been isolated. (In six of the blood-cultures obvious contaminations were recorded).

*B.—Bacteriological Investigations made Post-mortem in ten Cases of Rheumatic Fever (and Chorea).*

During the three years 1905-7 I have undertaken systematic examinations of ten persons dead of rheumatic fever, paying special attention to the cultivation of micro-organisms from the heart's blood, pericardial and pleural effusions and endocardial vegetations. Here, again, a considerable variety of culture media has been employed, and some of the cultures have been duplicated for purposes of anaerobic as well as aerobic growth. In the case of the "rheumatic" vegetations on the valves, these have in most instances been ground up in a mortar and examined by films made direct from the macerated tissue as well as by cultures made from the same material.

*(i.) Positive Results (three cases).*

TABLE III.

No.	Sex.	Age.	Nature of Disease.	Results of Bacteriological Examination.
1	Female	16	Second attack of rheumatic fever, very severe; acute endocarditis; acute pericarditis with effusion; acute myocarditis; old mitral disease.	Heart's blood, sterile. Pericardial fluid, scanty growth of streptococcus longus. Vegetations, sterile.
2	"	7	Severe rheumatic fever; acute endocarditis and myocarditis; adherent pericardium with recent effusion.	Heart's blood, scanty growth of streptococcus brevis and medius. Pericardial fluid, sterile. Vegetations, sterile.
3	Male	15	Rheumatic fever; acute pericarditis with effusion; myocarditis; old mitral and aortic disease.	Heart's blood, sterile. Pericardial fluid, several colonies of streptococcus medius and brevis.

*(II.) Negative Results (seven cases).*

TABLE IV.

No.	Sex.	Age.	Nature of Disease.	Results of Bacteriological Examination.
1	Female	10	Rheumatic fever; acute endocarditis and pericarditis; adherent pericardium; pneumonia; pleural effusion.	All cultures sterile, except lung-juice which yielded pneumococci and streptococci.
2	"	24	Recurring acute rheumatism; old mitral and aortic disease; acute endocarditis and myocarditis.	All cultures sterile.
3	"	11	Severe chorea; acute endocarditis and pericarditis; acute dilatation of heart.	All cultures sterile.
4	"	20	Old rheumatic valvular disease, aortic and mitral; recent mitral tricuspid and aortic endocarditis; acute myocarditis.	Heart's blood, sterile. Vegetations yielded a few colonies of a coliform bacillus.

TABLE IV.—*continued.*

No.	Sex.	Age.	Nature of Disease.	Results of Bacteriological Examination.
5	Male	7	Severe acute rheumatism with endocarditis and pericarditis; pleural effusion; nodules.	All cultures, including pleural fluid, sterile.
6	Female	12	Recurring acute endocarditis and pericarditis; death from acute myocarditis.	All cultures sterile.
7	"	24	Rheumatic fever; old and recent endocarditis, mitral, tricuspid, and aortic.	All cultures sterile.

In ten fatal cases of acute rheumatism, therefore, micro-organisms were isolated post-mortem in three cases only. The growths were in all these cases scanty, and in each of them at least one of the inflamed tissues examined yielded no growth at all. As seen, all the micro-organisms isolated were streptococci. Amongst the negative cases there were several that gave excellent opportunities for investigation, in that the disease was in them well-marked, and care was taken to obtain the post-mortem examination whilst the body was still warm. The absence of any bacteria in the culture tubes becomes for these reasons all the more striking.

*C.—Blood Cultures Undertaken During Life in Thirty Cases of Malignant Endocarditis.*

During the three years 1905–7 I have had opportunities of examining the blood bacteriologically during life in thirty patients suffering from malignant endocarditis. The technique adopted was that already referred to. For the sake of convenience the cases yielding positive results are classified in the following table, so that those from which similar micro-organisms were isolated are grouped together.

*(i.) Positive Results (Thirty-seven Cultures in Twenty-seven Cases).*

TABLE V.

No.	Sex.	Age.	Previous occurrence of Rheumatism, &c.	Duration of illness before Culture.	Duration of illness after Culture.	Micro-organism Isolated.
1	Male	13	Two attacks rheumatic fever, last two years before this illness.	Three months	Two months	Streptococcus
1a	"	"	Same case, culture repeated.	Four months	One month	"
2	"	12	No rheumatism, no previous illness.	Ten days	Three days	"
3	"	33	No rheumatism, malaria some years previously.	Seven weeks	One week	"
4	Female	26	No rheumatism, Graves' disease immediately preceding this illness.	Three months	Four months	"

TABLE V.—*continued.*

No.	Sex.	Age.	Previous occurrence of Rheumatism, &c.	Duration of illness before Culture.	Duration of illness after Culture.	Micro-organism Isolated.
5	Male	52	No rheumatism, syphilis some years previously.	About three months.	Two months	Streptococcus.
6	"	44	Rheumatic fever 19 years previously.	About two months.	One month	"
7	Female	17	Rheumatic fever eight years previously.	About four months.	Two months	"
8	Male	34	No rheumatism ..	About six months.	Two weeks	"
8a	"	"	Same case, culture repeated.	—	One week	"
9	"	28	Rheumatic fever 10 years previously.	About five months.	Unknown	"
10	"	30	Rheumatic fever five years previously.	Four months	Six weeks	"
11	Female	31	Rheumatic fever 14 years previously.	About four months.	Seven weeks	"
11a	"	"	Same case, culture repeated.	—	Five weeks	"
11b	"	"	Same case, culture repeated.	—	Three weeks	"
12	Male	39	No previous disease known.	About nine months.	Two months	"
12a	"	"	Same case, culture repeated.	—	Three weeks	"
13	"	34	Three attacks rheumatic fever last six years before this illness.	Six weeks	Unknown	"
14	Female	16	Rheumatic fever two years previously, several subacute attacks since.	Indeterminate	Three months	"
15	Male	34	No rheumatic fever, developed "athlete's heart" 10 years previously.	Two months	Four months	"
16	"	30	No rheumatism ..	About three months.	Two months	"
17	Female	24	No rheumatism ..	About three months.	Three weeks	"
18	Male	31	Rheumatic fever 13 years and four years previously.	Ten weeks	Six weeks	B. influenza.
18a	"	"	Same case, culture repeated.	—	Four weeks	"
18b	"	"	Same case, culture repeated.	—	Two weeks	"
18c	"	"	Same case, culture repeated.	—	Four days	"
19	"	13	No rheumatism or other illness.	Three weeks	Five weeks	"
19a	"	"	Same case, culture repeated.	—	One week	"
20	"	"	Rheumatic fever 27 years previously.	Four months	Two weeks	"
21	Female	35	Rheumatic fever 10 years previously.	About two months.	Two months	"
22	Male	37	No known illness before this.	Two months	Unknown	"
23	"	10	Rheumatic fever, immediately followed by pneumonia and empyema.	Seven weeks	Two weeks	Pneumococcus.
24	"	28	No rheumatism ..	Three weeks	Ten days	"
25	"	15	No rheumatism ..	Six weeks	Two weeks	"
26	"	21	No rheumatism ..	Four weeks	Four weeks	Gonococcus.
26a	"	"	Same case, culture repeated.	—	Two weeks	"
27	Female	21	No acute rheumatism	About 15 months.	Two weeks	Staphylococcus albus.

## (ii.) Negative Results (Three Cultures in Three Cases).

TABLE VI.

No.	Sex.	Age.	Previous occurrence of Rheumatism, &c.	Duration of illness before Culture.	Duration of illness after Culture.	Micro-organism Isolated. Post-mortem.
1	Male	23	No rheumatism ..	About six weeks.	Two weeks	Streptococcus faecalis.
2	"	37	Rheumatic fever 20 years previously.	About nine months.	One month	Pneumococcus.*
3	"	39	No rheumatism ..	Two months	Two weeks	Gonococcus.

\* This patient developed lobar pneumonia and pericarditis four days before death; the condition was probably a terminal pneumococcal infection. It is likely that the original micro-organism causing the chronic endocarditis was not isolated post-mortem.

It is seen from these results that, with the particular technique adopted, blood cultures undertaken *in vivo* give positive results in 90 per cent. of cases of malignant endocarditis. By the kindness of Dr. F. W. Andrewes I am able to add to these twenty-seven positive results eight others obtained by himself and his colleagues in the pathological department of St. Bartholomew's Hospital during the year 1907.

## D.—Eight Additional Positive Results of Blood Cultures Undertaken during Life in Cases of Malignant Endocarditis.

TABLE VII.

No.	Sex.	Age.	Previous occurrence of Rheumatism, &c.	Duration of illness before Culture.	Duration of illness after Culture.	Micro-organism Isolated.
28	Female	25	No rheumatism, illness followed operation for mastoid disease.	One week	One week	Streptococcus.
29	Male	52	No rheumatism ..	At least three months.	One month	"
30	Female	18	Rheumatic fever two years previously.	About six weeks.	Unknown	"
31	Male	30	Rheumatic fever 12 years previously.	About four months.	Two months	"
32	Female	36	Rheumatic fever 10 years previously.	About three months.	One month	"
33	"	32	No rheumatic fever, pyosalpinx preceded this illness.	Two months	Two weeks	"
34	"	35	Rheumatism 10 years previously.	Three months	Four days	B. influenzae.
35	"	27	Rheumatic fever 10 years previously.	About two months.	One week	Pneumococcus.

These thirty-five positive results constitute a series sufficiently large to justify an analysis of the micro-organisms obtained. In twenty-three of the cases post-mortem examinations were made, and in all of these the diagnosis of malignant endocarditis was confirmed, and the micro-organisms either cultivated or demonstrated in film preparations.

E.—*Analysis of thirty-five Positive Results of Blood Culture During Life in Cases of Malignant Endocarditis with regard to the Micro-organism Isolated.*

TABLE VIII.

1	Streptococcus ... ..	Isolated in 23 cases.	
2	B. influenzae ... ..	" " 6 "	
3	Pneumococcus ... ..	" " 4 "	
4	Gonococcus ... ..	" " 1 case.	
5	Staphylococcus albus ...	" " 1 "	

Malignant endocarditis is seen to be pre-eminently a form of streptococcal infection, for streptococci occur in over 65 per cent. of this series of cases. If the pneumococcus be regarded as a type of streptococcus this percentage rises to over 77. The high place occupied in this series by Pfeiffer's bacillus is in harmony with the view previously taken by the writer with regard to the prevalence of this micro-organism in malignant endocarditis. From a patient admitted to St. Bartholomew's Hospital, under the care of Dr. Samuel West (No. 34), Dr. Hugh Thursfield recently isolated a short Gram-negative bacillus having all the characters of Pfeiffer's bacillus and yielding cultures similar to those obtained by myself in four previous cases. I have since isolated this micro-organism from a fifth case of chronic endocarditis by blood culture *in vivo* (No. 22). No other instances of the cultivation of the influenza bacillus from the blood during life appear to have been reported. The fact that the gonococcus was the micro-organism present in the endocardium in one of my three negative cultures suggests that this coccus may not seldom be overlooked during life on account of the difficulties connected with its growth. The single case of endocarditis associated with staphylococcus albus is interesting in that its duration was certainly more than eighteen months. The absence of staphylococcus aureus from this series is due to the fact that these cases are, with two exceptions, drawn from medical, and not surgical, sources. Thus in cases of osteomyelitis developing endocarditis the commonest micro-organism to be found in the heart valves and in the blood stream is undoubtedly *S. aureus*. But in such cases, and in other cases where a focus of pyogenic infection exists prior to the development of malignant endocarditis, the latter condition is an incident in the pyæmia rather than the source of it. This was so in the two exceptions already referred to. They are Nos. 28 and 33. In the former the fungating endocarditis present was part of an acute streptococcal pyæmia following a radical operation for mastoiditis. In the latter it was secondary to pyosalpingitis.

*The Characters of the Streptococci Isolated from Three Cases of Rheumatic Fever after Death, and from Twenty-three Cases of Malignant Endocarditis During Life.*

Various strains of streptococci being by far the commonest micro-organisms associated with rheumatic fever and with

malignant endocarditis, it is in connection with these cocci that bacteriological interest in these two diseases chiefly lies.

Until recently no adequate means of differentiation of the streptococcus group of micro-organisms existed. (For a criticism of the various attempts at such differentiation see "A Study of the Streptococci pathogenic for man," by Andrewes and Horder, in *The Lancet*, September 15th, 22nd, and 29th, 1906). In a paper contributed to the Medical Officer's Report for 1903-4, however, Dr. Mervyn H. Gordon recorded his researches into certain metabolic reactions given by streptococci derived from different sources, using various chemical substances as foodstuff for the cocci. Gordon selected seven substances as being of special value for differential purposes: saccharose, lactose, raffinose, inulin, salicin, coniferin, and mannite. He added to these the clotting of milk and the reduction of neutral red under anaerobic conditions. Gordon used these tests in the examination of 300 strains of streptococci isolated from normal saliva. Omitting the coniferin reaction, Dr. A. C. Houston investigated 300 strains of streptococci derived from normal fæces on similar lines. His results are recorded in the same volume (XXXIII.) of the Medical Officer's Reports. The technique of these tests and the main results obtained, were summarised by Gordon in *The Lancet*, November 11th, 1905, p. 1,400.

With these nine tests suggested by Gordon as our chief guide Dr. Andrewes and I proceeded to investigate more than 200 strains of streptococci and pneumococci derived from human disease-processes. Our results are embodied in the paper already referred to. We were able to distinguish five main types of streptococci pathogenic for man having characters sufficiently distinct, despite the existence of many intermediate forms, to deserve type names. These five types are as follows:—

TABLE IX.

Types.	Reactions with Gordon's nine tests.								Growth on gelatin at 20° C.	Morphology.	Pathogenesis for mouse.	
	Milk clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.				Mannite.
1. Streptococcus pyogenes	-	-	+	+	-	-	±	-	-	+	Longus	+
2. Streptococcus salivarius	+	±	+	+	±	-	-	-	-	±	Brevis	-
3. Streptococcus anginosus	+	±	+	+	-	-	-	-	-	±	Longus	+
4. Streptococcus faecalis ..	+	+	+	+	-	-	+	+	+	+	Brevis	-
5. Pneumococcus .. ..	±	-	+	+	+	±	-	-	-	-	Brevis	+

Applying these tests to the streptococci isolated from three patients dead of rheumatic fever, and from twenty-seven cases of malignant endocarditis during life, the following are the results.

*Characters of the Streptococci isolated post-mortem in three cases of rheumatic fever.*

TABLE X.

No.	Type.	Milk clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth on gelatin at 20° C.	Morphology in broth.	Pathogenicity for mouse.
1. Colony (a)	<i>S. pyogenes</i> ..	-	-	+	+	-	-	+	-	-	Good	Longus	+
Colony (b)	<i>S. anginosus</i> ..	+	+	+	+	-	-	-	-	-	None	"	-
2. Colony (a)	<i>S. pyogenes</i> ..	-	-	+	+	-	-	+	-	-	Good	Longus	?
Colony (b)	<i>S. anginosus</i> ..	+	+	+	+	-	-	-	-	-	None	"	?
Colony (c)	"	+	-	+	+	-	-	-	-	-	"	"	?
3. Colony (a)	<i>S. anginosus</i> ..	+	-	+	+	-	-	+	+	-	Feeble	Medius	?
Colony (b)	"	-	-	+	+	+	-	-	-	-	None	Longus	?
Colony (c)	"	-	-	+	+	+	-	-	+	-	"	"	?
Colony (d)	"	-	-	+	-	+	-	+	+	-	"	"	?

No special set of reactions, therefore, characterised these streptococci. The fact that in each of the cases more than one variety was present suggests that the cocci may in all cases have been of the nature of terminal infections.

*Characters of the Streptococci isolated by blood culture during life in twenty-three cases of malignant endocarditis.*

TABLE XI.

No.	Type.	Milk clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth in gelatin at 20° C.	Morphology in broth.	Pathogenicity for mouse.
1	<i>S. pyogenes</i> .. ..	-	-	+	+	-	-	+	-	-	Good	Longus	?
2	" .. ..	-	-	+	-	-	-	+	-	-	"	"	+
28	" .. ..	-	-	+	+	-	-	+	-	-	"	"	+
3	<i>S. salivarius</i> .. ..	+	+	+	+	+	-	-	-	-	None	Brevis	-
4	" .. ..	+	+	+	+	-	-	-	-	-	Feeble	"	-
5	" .. ..	+	-	+	+	-	-	+	+	-	"	"	?
6	" .. ..	+	+	+	+	-	-	+	-	-	Fair	"	?
7	" .. ..	+	-	+	+	+	+	+	-	-	None	Medius	?
29	" .. ..	+	+	+	+	+	-	+	+	-	Good	Brevis	-
30	" .. ..	+	+	+	+	-	+	+	+	-	Feeble	"	?
31	" .. ..	+	-	+	+	+	+	+	+	-	None	Medius	?

TABLE XI.—continued.

No	Type.	Milk clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Sallein.	Conferin.	Mannite.	Growth in gelatin at 20° C.	Morphology in broth.	Pathogenicity for mouse.
8	<i>S. anginosus</i> .. ..	+	+	+	+	+	-	-	-	-	Good	Longus	+
9	" .. ..	+	+	+	+	+	-	-	-	-	"	"	?
10	" .. ..	+	-	+	+	-	-	-	-	-	Feeble	"	?
11	" .. ..	-	-	+	+	+	-	-	-	-	None	"	-
12	" .. ..	-	-	+	+	+	-	-	-	-	"	"	?
33	" .. ..	-	-	+	+	+	-	+	+	-	Good	"	?
13	<i>S. faecalis</i> .. ..	+	+	+	+	-	-	+	+	+	Good	Brevis	-
14	" .. ..	+	-	+	+	+	+	+	-	+	"	Longus	-
15	" .. ..	+	-	+	+	+	+	+	-	+	"	"	?
16	" .. ..	+	-	+	+	+	-	+	-	+	"	Medius	?
17	" .. ..	+	+	-	+	-	-	+	+	+	"	"	?
33	" .. ..	+	-	+	+	+	+	+	?	+	"	Longus	?

A study of the above table shews clearly that the type of streptococcus most often found in cases of malignant endocarditis is not the highly pathogenic streptococcus of suppurative processes (*S. pyogenes*; *Syn. S. erysipelatos*), nor the equally virulent pneumococcus, but one or other of three types of streptococci that are more closely allied to the "saprophytic" streptococci of the alimentary tract. Of these three types that to which, in our differential study of the disease-producing streptococci, Andrewes and I gave the name *S. anginosus*, shews the most pathogenicity for the mouse. And this type is less often represented than are the salivary and the faecal types. Streptococcal malignant endocarditis, then, may be regarded as an infection of the endocardium by micro-organisms of low virulence, of the types of the streptococci found in normal saliva and in normal faeces. They are for the most part short chained cocci, not pathogenic for mice, and yielding a set of bio-chemical reactions that mark them off quite sharply from streptococcus pyogenes. These remarks apply specially to the chronic cases of streptococcal endocarditis; but such cases are far commoner than the acute and fulminating cases of malignant endocarditis. In these latter the micro-organism present is, if a streptococcus, of the type *S. pyogenes*; or it may be the pneumococcus; but it is as often not streptococcal at all, *e.g.*, staphylococcus aureus. And, as already mentioned, the acute cases are apt to be associated with suppurative foci elsewhere in the body (as in No. 28, Table VII.). It is because of the close association of these causal streptococci in malignant endocarditis with the common "saprophytes" of the mouth and intestine that I have dealt only

with the cocci isolated from the blood during life in these cases. If obtained post-mortem only it might reasonably be argued that some of them, at least, were possibly of the nature of terminal infections, or even of post-mortem invasions. I have, therefore, omitted any reference to a considerable number of post-mortem observations, although these confirm in every way the main conclusions arrived at.

### SECTION III.

#### EXPERIMENTAL INVESTIGATION.

It having been established by the foregoing clinical and pathological observations that the micro-organisms most commonly associated with malignant endocarditis are streptococci of the salivary and faecal types, the question arose: are the "saprophytic" streptococci of the human alimentary canal capable of setting up endocarditis under the conditions of animal experiment? Before submitting this question to investigation it was first determined whether a strain of streptococcus belonging to one or other of these types, when isolated by blood culture from a patient suffering from chronic malignant endocarditis, could be made to reproduce the disease in the rabbit. The streptococcus numbered 6 in Table XI., having been isolated a week previously from a case of malignant endocarditis, was taken as the test organism. The reactions given by this streptococcus were as follows:—

TABLE XII.

Litmus Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth on Gelatin at 20° C.	Morphology in Broth.
Acid.	Clot.										
+	+	+	+	+	-	-	+	-	-	Fairly good	Brevia. Uniform turbidity.

The strain was thus the type form of *S. salivarius*.

*Experiment I.*—To determine whether the salivary type of *Streptococcus*, obtained by blood culture from a case of chronic malignant endocarditis can reproduce this disease by intravenous injection in the rabbit.

Rabbit W, weighing 2,170 grammes. First injection on December 22nd, of 10 cc. of a 48 hours' broth culture of the streptococcus made from the single colony yielding the reactions in Table XII. No ill-effects were observed. Ten days later the injection was repeated under similar conditions. Again, no ill-effects were observed. The rabbit died on February 8th, 48 days after the first injection. A few days before death it was noticed that the belly was large; this was thought to be due to over-eating of green

food. The *weight* of the animal had sunk from 2,170 to 1,733 grammes ten days before death. It rose again to 1,900 grammes before death, the increase of weight evidently being due to the accumulations of oedema fluid found in the serous membranes post-mortem. The *leucocyte counts* made during the course of the experiment showed a fall during the first 24 hours after the first injection from an initial 6,500 to 3,200. The white cells rose steadily after this time until they reached 28,000 the day after the second injection. The number then fell a second time, reaching 4,800 before death. The *red blood cells* shewed a marked fall during the first 24 hours, from 5,800,000 to 2,900,000, rising steadily during the next few days and remaining at about 4,000,000 throughout the experiment. The changes in the *hæmoglobin* percentage was proportionate to those in the red cells.

*Post-mortem examination.*—The flanks are noticed to bulge. The thoracic and abdominal walls are seen to be oedematous when cut. The *peritoneum* contains 20 cc. of slightly blood-stained fluid. The right *pleura* contains 25 cc., the left pleura 15 cc., of straw-coloured fluid. All these effusions clot rapidly on removal and form a solid coagula, so that the containing vessels can be inverted without their contents falling out. Both *lungs* are pale and collapsed. The *liver* is very large and weighs 99 grammes; it shews obvious signs of chronic congestion, and a section through it reveals a distinct "nutmeg" character. The *kidneys* are cyanotic and indurated; a small hæmorrhage (? infarct) is present at the upper pole of the left kidney. The *pericardium* contains about 1 cc. of straw-coloured fluid and a few flakes of fibrin; the surface of the membrane is slightly rough near the origin of the large vessels. The *heart* is considerably enlarged, the chamber most affected being the left auricle, which is much dilated; the right ventricle is also dilated. On opening the left auricle the lower half of the cavity is seen to be filled by pinkish, rounded masses which completely hide the mitral orifice, and prevent this latter being found with a probe. On cutting open the left ventricle from the apex and extending the cut through the mitral ring the masses are seen to be growing from the auricular surface of the valve cusps and protruding upwards into the auricle. The wall of the auricle is free from vegetations. So also are the tricuspid, aortic, and pulmonary valves. The appearance of the mitral vegetations is shewn in *Plate I*.

*Bacteriological examination.*—The *heart's blood* yielded a pure culture of streptococci. So also did the mitral vegetations. On testing the streptococcus obtained from the vegetation the reactions obtained in the carbohydrate media were similar to those given by the streptococcus used for the intravenous injections (see *Table XII*).

It was thus shewn that a streptococcus obtained by blood-culture during life from a case of malignant endocarditis, and yielding reactions characteristic of "saprophytic" salivary streptococci was capable of reproducing the disease in the rabbit. It may be noted that the picture of the disease produced experimentally was very close indeed to the clinical picture. The extreme dilatation of the left auricle, the stenosis of the mitral orifice, the multiple serous

effusions, the "nutmeg" liver, the congested kidneys,—all these appearances were well-marked in the body of the patient as seen post-mortem.

Attention was next turned to the question of the production of endocarditis by the use of "saprophytic" streptococci obtained from the human alimentary canal. For the purpose of the experiments undertaken in dealing with this question normal fæces and normal saliva were taken. The fæces and the saliva were plated out on agar after appropriate dilution, and single colonies of streptococci were picked out on the second day, sub-cultivated, and tested for their reactions in the manner already dealt with.

*The production of endocarditis in rabbits by the use of fæcal streptococci.*

Three colonies of streptococci were isolated from an agar plate made in the manner described above, the normal fæces being used. The reactions given by these three colonies were as follows:—

TABLE XIII.

Colony.	Litmus Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth on Gelatin at 20° C.	Morphology in broth.
	Acid.	Clot.										
A .. ..	+	+	-	-	+	-	-	+	+	-	Rapid and abundant.	Brevis uniform turbidity.
B .. ..	+	+	-	-	+	-	-	-	-	-	Rapid and abundant.	Brevis uniform turbidity.
C .. ..	+	+	+	-	+	-	-	+	+	+	Rapid and abundant.	Brevis uniform turbidity.

The streptococcus in colony A and in colony B respectively were of the salivary type. That in colony C was of the fæcal type. Broth cultures of each of these three colonies were prepared and, after 48-hours' growth, these were injected into rabbits intravenously.

*Experiment II.—To determine whether "saprophytic" streptococci obtained from normal human fæces can produce endocarditis when injected into rabbits intravenously.*

Rabbit A, weighing 1,910 grammes. First injection of 10 cc. of broth culture of colony A on December 14th. Second injection of 10 cc. of broth culture of colony A on December 20. Third injection of 10 cc. of broth culture of colony A on December 31. No immediate ill-effects were seen after either of these injections. The animal died on January 10, 27 days after the first injection. The rabbit's weight fell steadily throughout the experiment. The



**PLATE I.**—Mitral Endocarditis in the rabbit, produced by intravenous injection of *Streptococcus salivarius* obtained from the blood of a patient suffering from ulcerative Endocarditis.



**PLATE II.**—Mitral Endocarditis in the rabbit, produced by intravenous injection of *Streptococcus faecalis* isolated from the normal human intestine.



**PLATE III.**—Aortic Endocarditis in the rabbit, produced by intravenous injection of *Streptococcus salivarius* isolated from the normal human mouth.

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number of *leucocytes* rose from 4,400 at the beginning of the experiment to 13,200, 24 hours after the first injection, then fell to 4,400 on the fifth day. The day after the second injection the number had risen to 10,800. The count remained near this figure until the third injection after which a fall occurred. The *red cells* shewed only a slight hæmolyseis, the number varying but little throughout the course of the experiment. A *blood culture* undertaken two days after the first injection gave three colonies of streptococci per 1/100 cc. of blood and about 23 colonies of streptococci per 1/10 cc. of blood, *i.e.*, about 200 cocci per cc. of blood.

*Post-mortem examination.*—The *lungs* are œdematous and congested. There are no exudations into pleura, pericardium or peritoneum. No joint changes are observed. The *heart* is somewhat dilated, and this is especially marked in the left ventricle. A massive form of *mitral endocarditis* is present, the orifice of the mitral valve being blocked by a number of large vegetations which also occupy a considerable part of the chamber of the left auricle. There is no "mural" endocarditis. The aortic, pulmonary and tricuspid valves are natural. Plate II. shews the morbid appearances seen in this rabbit's heart.

*Bacteriological examination.*—Both the heart's blood and the mitral vegetations yielded, on cultivation, a pure culture of streptococci in large numbers. The streptococcus thus recovered gave identical reactions with those obtained with the micro-organism as isolated from the fæces (*see* Table XIII).

*Experiment III.—To determine the same question.*

Rabbit B, weighing 1,845 grammes. Similar injections were given, and at the same interval of time as in Experiment II., using colony B. No immediate ill-effects.

The *number of leucocytes* rose from 6,000 before the first injection to 18,400 after it, from 8,000 before the second injection to 14,000 after it, and from 8,800 to 30,000 after the third injection. This large response on the part of the leucocytes compares strikingly with the much more moderate leucocytosis seen in the case of rabbit A and undoubtedly indicates a higher degree of resistance to infection by the streptococcus. (This is in accordance with the results—for rabbit B shewed no development of endocarditis or other local mischief due to the streptococcus.) This leucocyte count had returned to normal (5,200) a month after the first injection. A fourth injection was then given. Twenty-four hours later the leucocyte count shewed no increase (5,600). This gave strong presumptive evidence that no pathogenic effect was now being exercised by the micro-organism.

The *red cells* in the case of this animal fell by nearly 40 per cent. after the first injection (4,720,000 to 2,912,000) and by over 30 per cent. after the second injection (4,012,000 to 2,700,000). But they were scarcely affected by the third injection (5,860,000 to 5,500,000) nor by the fourth (6,000,000 to 5,720,000).

This high degree of hæmolysis occurring after the first and after the second injections contrasts also with the very slight effect produced upon the red corpuscles by the injections in the case of rabbit A. A *blood culture* performed 24 hours after the fourth injection shewed three specimens of 1/100 cc., 1/1000 cc., and 1/10 cc. of blood to be all of them sterile.

This result of blood culture forms a third very striking contrast with rabbit A.

The resistance in the case of rabbit B to infection by the particular fæcal streptococcus selected for the injections appeared thus to be unassailable, since the blood or other tissues had become capable of disposing of these enormous quantities of cocci without any demonstration on the part of the animal of recognisable changes during the effort. Six weeks after the first injection this rabbit was killed by chloroform and examined post-mortem. Nothing abnormal was found in the heart or elsewhere. Cultivations made from the heart's blood proved to be sterile.

*Experiment IV.—To determine the same question.*

Rabbit C, weighing 1,460 grammes. Similar injections as in Rabbit A, but using colony C. No immediate ill-effects. The *leucocytosis* observed in the case of this rabbit was even greater than that seen in the case of rabbit B. From an initial count of 5,600 the figure rose to 36,000 after the first injection and after the second injection it rose from 7,200 to 26,000. The third injection failed to produce any rise at all (5,600) and the fourth also failed to increase the number of white cells. The *red blood cells* diminished more than 50 per cent. in number after the first injection (6,375,000 to 3,193,000), but they were scarcely at all affected by the subsequent three injections.

A *blood culture* undertaken 24 hours after the fourth injection shewed two specimens of 1/100 cc. and 1/10 cc. of blood respectively, both sterile. The similarity between the response made to the injections by this rabbit and by rabbit B was so close that it was predicted that no heart lesion had been produced. This prediction was verified by examination of the animal after killing it by chloroform eight weeks from the date of the first injection.

Out of three rabbits, therefore, injected intravenously with streptococci derived from the healthy human intestine, one developed fatal endocarditis.

*The production of endocarditis in rabbits by the use of salivary streptococci.*

From an agar plate which had been inoculated with diluted normal saliva three colonies of streptococci were isolated and sub-cultivated. They were found to give the following reactions:—

TABLE XIV.

Colony.	Litmus Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Conifein.	Mannite.	Growth on Gelatin at 20° C.	Morphology in broth.
	Acid.	Clot.										
D .. ..	+	+	-	+	+	+	-	+	?	-	No growth.	Medius.
E .. ..	+	+	+	+	+	+	-	-	?	-	Slow and slight.	Brevis to medius.
F .. ..	+	+	+	+	+	-	-	-	?	+	Slow and slight.	Brevis.

(The conifein re-action was not tried in the case of these colonies.)

The administration of the injections differed slightly from that adopted in the case of the intestinal streptococci. The injections were given every fourth day, and the amount of broth-culture injected upon each occasion was 5 cc. The injections were intravenous as before.

*Experiment V.*—To determine whether “saprophytic” streptococci derived from normal human saliva can produce endocarditis when injected into rabbits intravenously.

Rabbit D. First injection given on June 17th, using 5 cc. of a 24 hours’ growth in broth from colony D. The second injection was given on June 21st and the third on June 25th. On June 21st a small abscess, containing a large drop of curdy pus, was found at the site of inoculation on the ear. From this pus, on cultivation, a pure growth of streptococci was obtained, and the reactions given by the micro-organism were the same as those of colony D (see Table XIV.). This rabbit was found to be dead on July 1st, 15 days after the first injection.

*Post-mortem examination.*—The lungs, liver and spleen are all congested. There are no exudations into the serous cavities. The heart does not appear to be appreciably enlarged. There is a marked degree of endocarditis, of the fungating variety, affecting the aortic valves, from the cusps of which several large vegetations are seen to be sprouting into the orifice of the valve. A few minute vegetations are also seen upon the aortic aspect of one mitral cusp. The pulmonary and tricuspid valves are free from vegetations. The condition of the aortic valve is seen in Plate III.

*Bacteriological examination.*—Cultivations made from the heart’s blood and from the aortic vegetations gave in both instances pure growths of streptococci. On testing the streptococcus obtained from the vegetations it was found to have lost its raffinose reaction but in other respects it appeared identical with colony D as isolated from the saliva (see Table XIV.).

*Experiment VI.—To determine the same question.*

Rabbit E. Injections given as in Experiment V. but using colony E. This rabbit was alive and well three months after the first injection, but then it died. A post-mortem examination revealed no lesion in the heart or any other organ.

*Experiment VII.—To determine the same question.*

Rabbit F. Injections given as in Experiment V., but using Colony F. This animal was alive and well four months after the first injection. It was then used as the control animal in an entirely different set of experiments, and died as the result of this. Post-mortem examination showed no disease of the heart or pericardium.

In the case of salivary streptococci, therefore, as in the case of faecal streptococci, fatal endocarditis was set up in one out of three rabbits injected with the micro-organisms.

*Summary of results of these experiments.*—The so-called "saprophytic" streptococci of the human alimentary canal, whether obtained from the intestines or from the mouth, are capable, when injected into rabbits intravenously, of producing endocarditis. Susceptibility appears to vary in different rabbits. When it is high the injection of the streptococci produces but a mild response in the form of leucocytosis, and but a slight destruction of red blood corpuscles. When susceptibility is low the leucocytosis is very considerable, and the degree of hæmolytic is also marked. Again, when susceptibility is high the micro-organisms are recoverable from the blood stream by culture for some time after injection; when it is low they disappear very rapidly from the circulation.

COMPARISON OF THESE RESULTS WITH THOSE OBTAINED  
BY INTRAVENOUS INJECTION OF HIGHLY "PATHOGENIC"  
STREPTOCOCCI.

The marked clinical difference existing between cases of endocarditis, due to such virulent micro-organisms as streptococcus pyogenes and the pneumococcus on the one hand, and the salivary and faecal types of streptococcus on the other, were dealt with in Section II. It seemed a matter of importance to get a clear view of the experimental picture resulting from the use of these particular strains of streptococci for purposes of comparison. The production of acute endocarditis by intravenous injection of such highly pathogenic micrococci in rabbits appears to be difficult. Out of four rabbits injected with living pneumococci, and out of three rabbits injected with streptococcus pyogenes, with fatal results in all seven animals, no instance of endocarditis occurred.

The source of the pneumococcus used in these experiments was the heart's blood of a patient dead of lobar pneumonia on the fifth day of the disease. The source of the streptococcus

pyogenes (L) used in Experiment XII. was the peritoneal fluid obtained post-mortem from a case of septicæmia following ulcerative stomatitis; the streptococcus pyogenes (M) used in Experiment XIII. was isolated from the pus in a case of cellulitis of the finger. The bio-chemical reactions given by these micro-organisms were as follows :—

TABLE XV.

—	Litmus Milk.		Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth on Gelatin at 20° C.	Morphology in Broth.
	Acid.	Clot.										
Pneumococcus..	+	-	-	+	+	+	-	-	-	-	None	Brevis; uniform turbidity.
S. pyogenes (L)	+	-	-	+	+	-	-	+	-	-	Good	Longus;ropy deposit.
S. pyogenes (M)	+	-	-	+	+	-	-	+	-	-	"	Longus;ropy deposit.

The records of these seven experiments are as under :—

*Experiment VIII.—To determine the results following the intravenous injection of living Pneumococci in Rabbits and particularly whether such injection can be made to cause endocarditis.*

Rabbit G, weighing 2,155 grammes. On December 23rd, an intravenous injection consisting of 10 cc. of a 24 hours' culture of pneumococci in blood broth. In less than 24 hours this animal was found dead. Cultivations from the heart's blood yielded abundant growth of pneumococci. There were no lesions discovered in the heart or other organ.

*Experiment IX.—To determine the same question*

Rabbit H, weighing 2,625 grammes. On December 28th, an intravenous injection of 0.25 cc. of a 24 hours' blood broth culture of pneumococci was given. No ill effects were observed on December 29th. The 24 hours' growth on two blood-agar tubes was then injected. On December 30th, there were still no signs of illness, and a third injection of 4 cc. of a 24 hours' blood broth culture was given. On December 31st, the animal was noted to be ill. Its weight had fallen to 2,515 grammes. A blood culture showed 1/5 cc. of blood to contain very numerous colonies of pneumococci. The rabbit died on this day, the third after the first injection. The leucocyte count was 5,000 before the first injection; it rose to 15,000 the day following the first injection of blood broth culture, but fell to 5,200 the day after the second injection of colonies from the agar slopes. The day after the third injection of blood broth

culture it rose again, to 21,000. During this day, towards the end of which the animal died, the leucocytes rapidly diminished from this number to 16,000, and later to 8,000 two hours before death. The *red cells* numbered 4,000,000 before the first injection. The day following it they had increased to 5,500,000. They remained about this figure after the second and third injections, and were 5,600,000 two hours before death. The *hæmoglobin* percentage showed a decline from 80 to 70 the day after the first injection. No change followed the second injection, but the percentage fell to 60, and later to 57, the day following the third injection; it was this last figure two hours before death. *Post-mortem* examination showed the spleen to be enlarged, but no other abnormality was noted.

*Experiment X.—To determine the same question.*

Rabbit J, weighing 1,845 grammes. On January 1st, an intravenous injection of 1 cc. of a 24 hours' blood broth culture of pneumococci was given. This animal was obviously ill next day; its *weight* had fallen to 1,735 grammes. On the third day this had further sunk to 1,710 grammes, and the rabbit was collapsed. It was found dead on the fourth day. The *leucocyte count* showed a gradual fall throughout the experiment; from an initial count of 6,800, the number was 4,000 on the second day, and 2,400 on the third day. The *red cells* showed practically no variation. Commencing at 4,760,000 they rose to 5,000,000 on the second day, and fell to 4,400,000 on the third day. The *hæmoglobin* fell from 70 per cent. at the start to 60 per cent. on the third day. *Post-mortem* examination showed some enlargement and congestion of the spleen but no other abnormality.

*Experiment XI.—To determine the same question.*

Rabbit K, weighing 1,970 grammes. On January 5th, an injection of 2 cc. of a 24 hours' blood broth culture of pneumococci was given. This animal was found dead two days later. In the case of this rabbit observations were made at frequent intervals during the 12 hours immediately following the injection, with the following results. The *weight* fell to 1,875 grammes in the 12 hours (a loss of 5 per cent.). The initial *leucocyte count* was 3,000. Four hours after injection the count was 5,700; seven hours after it was 4,500; eleven hours after it was 5,600 (a meal of bread was given two hours before this last count). There was, therefore, no appreciable leucocytosis following the injection. Neither the *red cells* nor the *hæmoglobin* showed any appreciable change in the twelve hours. *Blood cultures* showed a steady increase in pneumococcal content of the blood:—

Two hours after injection	1/100 cc. blood =	1 colony.
	1/100 cc. " =	3 colonies.
Four hours after injection	1/10 cc. " =	25 "
	1/100 cc. " =	1 colony.
	1/100 cc. " =	8 colonies.
	1/10 cc. " =	90 "

Seven hours after injection	1/100 cc.	„	=	33	„
	1/100 cc.	„	=	45	„
	1/10 cc.	„	=	150	„
Eleven hours after injection	1/100 cc.	„	=	50	„
	1/100 cc.	„	=	150	„
	1/10 cc.	„	=	colonies un-	countable.

*Post-mortem* examination revealed no gross lesion of any organ.

*Experiment XII.*—To determine the result following the intravenous injection of living cultures of *Streptococcus pyogenes* in Rabbits and particularly whether such injection can be made to cause endocarditis.

Rabbit L, weighing 2,345 grammes. On December 29th, an injection of 10 cc. of a 24 hours' broth culture of *Streptococcus pyogenes*, colony L (*see* Table XV.). This animal was found dead on the third day. No appreciable change was noted in the *leucocyte count* nor in the number of *red blood cells* on the day following the injection. The *hæmoglobin* percentage had fallen from 74 to 57, and the animal's weight had fallen to 2,265 grammes (a loss of 3·4 per cent.). *Post-mortem* examination showed no abnormality except some enlargement of the spleen. Cultures of the heart's blood yielded streptococci in large numbers.

*Experiment XIII.*—To determine the same question.

Rabbit M, weighing 1,530 grammes. On January 1st, an injection of 1 cc. of a 24 hours' broth culture of *Streptococcus pyogenes*, colony M (*see* Table XV.) was given. There were no ill effects noticed during the first five days. Then the animal was seen to be ill, and to be losing flesh. On the tenth day the back was stiff, and the wasting had become marked. The stiffness increased, and the rabbit died in an emaciated condition on the twenty-first day of the experiment. The *weight* fell gradually during the first five days, reaching 1,465 grammes (a loss of 4·2 per cent.) on the fifth day. It then fell more rapidly until, on the twenty-first day, it reached 1,047 grammes (a loss of 31·5 per cent.). The *leucocyte count* rose from 14,000 to 26,000 on the second day, fell to 16,000 on the third day, and to 12,000 on the fifth day. It was still 12,000 on the twelfth day, but fell to 8,400 on the fourteenth day. Slight decrease occurred both in the *red cells* and in the *hæmoglobin* throughout the course of the experiment. The *blood cultures* gave the following results:—

One day after injection	1/100 cc.	blood	=	150 colonies.
	1/10 cc.	„	=	colonies un-
				countable.
Two days after injection	1/100 cc.	„	=	3 colonies.
	1/10 cc.	„	=	20
		„	=	100
Three days after injection	1/100 cc.	„	=	150
	1/100 cc.	„	=	colonies un-
	1/10 cc.	„	=	countable,

Four days after injection 1/100 cc. blood = 107 colonies.  
 1/100 cc. " = 200 "  
 1/10 cc. " = colonies uncountable.

Eleven days after injection 1/100 cc. " = sterile.  
 1/100 cc. " = "  
 1/25 cc. " = "

*Post-mortem examination.*—The animal is much wasted. There are several foci of suppuration, the pus being everywhere very thick. A large abscess exists near the caecum, behind the peritoneum. Smaller abscesses are present in the mesenteric glands and in the spleen. Many small abscesses are seen in the vertebral muscles. The heart valves are natural. *Cultures* and films prepared from the pus of the spinal abscesses shew pure infection by streptococci. Streptococci are also recovered from the heart's blood. From the abscess near the caecum the cultures shew a mixture of *B. coli* and streptococci.

#### REVIEW of the EXPERIMENTS with VIRULENT STRAINS of STREPTOCOCCI.

It will be seen from consideration of these experiments that the injection of the pneumococcus or of streptococcus pyogenes into the blood-stream of rabbits leads either to a rapidly fatal condition of septicæmia without localisation of the infecting elements, or to a more slowly fatal condition of pyæmia, in which the blood-stream is comparatively free from micro-organisms but in which abscess formation is a marked feature. In experiments VIII. and XII. the dose of pneumococci and of streptococcus pyogenes respectively was known beforehand to be far above that which is lethal for the rabbit, but the dose was selected so that the results should form a strict comparison with those obtained by the use of the "saprophytic" streptococci of the preceding set of experiments. With doses as lethal as these death occurs so quickly that there is little time for making observations. If the time be extended by choosing a dose which is nearer to the minimal lethal dose, so that fuller observations can be made, it is found that in the case of the pneumococcus there is very slight response on the part of the leucocytes and only slight hæmolysis. The one striking event (as seen in Experiment XI.) is the rapid and progressive increase in the number of micro-organisms present in the blood up to the moment of death. In Experiment XIII. the animal lived for three weeks after the injection of a very small dose of streptococcus pyogenes, and eventually died in a condition characterised by multiple abscesses. Here the leucocytosis was considerable. But the red blood cells underwent no such diminution as was seen in the case of the "saprophytic" streptococci. The blood cultures revealed an initial decrease in the number of micro-organisms per cc. of the blood, then a considerable rise, and later, with the localisation of the streptococci in the tissues, the blood became (so far as the cultures were concerned) sterile. In none of these seven animals, as already stated, did endocarditis occur, although all of them succumbed to the infecting streptococci.

## SECTION IV.

## GENERAL CONCLUSIONS.

I.—*Summary of Results.*

The practical results of this particular investigation into the micro-organisms associated with acute rheumatism and malignant endocarditis are as follows:—

(i.) Cultivation of the blood bacteriologically during life in cases of rheumatic fever (whether complicated or not by chorea, endo- and peri-carditis and pleurisy) fails to discover any micro-organism. This negative result holds good even when considerable quantities of blood are withdrawn (up to 10 cc.) and when every known method of encouraging bacterial growth is employed.

(ii.) Cultivations of the heart's blood, endocardial vegetations, pericardial and pleural exudates in fatal cases of rheumatic fever fail to discover any micro-organism in the majority of cases. Examination of these materials directly by films is also in most cases negative.

(iii.) In the few cases in which micro-organisms are isolated from such post-mortem materials the growth is often scanty and consists of streptococci having no special features, but showing different reactions in different cases and sometimes different reactions in the same case.

(iv.) Cultivation of the blood bacteriologically during life in cases of malignant endocarditis, provided the technique of the examination is thorough, yields positive results in 90 per cent. of cases.

(v.) The micro-organisms thus obtained are not of the nature of terminal infections, for they may be cultivated from the blood early in the course of the illness, in some instances several months before death. Nor are they incidental infections, of transient character, for they may be cultivated repeatedly from the same case at varying intervals, and are found to yield the same reactions each time they are isolated. They are causal elements in the disease.

(vi.) Of the micro-organisms present in the blood of persons suffering from malignant endocarditis, those occurring most frequently are the streptococci, which are found in over 77 per cent. of the cases.

(vii.) Pfeiffer's bacillus ("B influenzae") is not an uncommon cause of malignant endocarditis, occurring in 6 per cent. of the cases.

(viii.) The streptococci are not, for the most part, of the type *S. pyogenes*. They are shown, by differential tests, to conform to the salivary and faecal types, as defined by the researches of Gordon and of Houston into the streptococci of the alimentary canal. They differ little, or not at all, from the "saprophytic" streptococci of the mouth and intestine. They are but feebly pathogenic for mice.

(ix.) These streptococci, of the types *S. salivarius* and *S. faecalis*, when isolated from cases of malignant endocarditis, are capable of reproducing the disease in all its features when injected intravenously into rabbits. The streptococci are recoverable from the lesions produced in these animals and are then found to yield the same series of bio-chemical and other reactions as the cocci originally isolated from the patients.

(x.) "Saprophytic" streptococci isolated from normal human saliva and from normal human faeces are also capable of causing acute and fatal endocarditis when injected intravenously into rabbits.

(xi.) Susceptibility appears to play an important part in the experimental production of malignant endocarditis in rabbits by the injection of "saprophytic" streptococci. In the animals whose endocardia become infected there is evidence of but slight interaction between the micro-organism and the host; the cocci can be recovered from the blood by culture for some time after the injection; there is only slight leucocytosis and slight hæmolysis. In the animals whose endocardia escape infection there is evidence of considerable interaction: the cocci rapidly disappear from the blood, the leucocytosis is high and there is a marked degree of hæmolysis.

(xii.) It is much easier to set up endocarditis in rabbits by the injection of faecal and salivary streptococci derived from the healthy human alimentary canal than by the injection of such highly pathogenic streptococci as *S. pyogenes* and the pneumococcus.

## II.—*The bearing of these Results upon our Knowledge of the Etiology of Rheumatic Fever and of Malignant Endocarditis.*

So far as these investigations have gone they support the recorded experience of those observers who have failed to isolate any causal micro-organism from the blood of patients suffering from rheumatic fever or from the blood and tissues of such patients after death.\* For the scanty growth of streptococci which was occasionally obtained post-mortem in patients dead of this disease cannot be regarded as other than of the nature of terminal infections.

There must be, of course, some explanation of the fact that one set of competent observers repeatedly succeeds in obtaining positive blood-cultures in rheumatic fever whilst another set as repeatedly fails. Only two explanations seem possible.

(i.) The technique of the blood culture may differ in the hands of the two sets of observers. That used by the positive school may be better adapted to the isolation of micro-organisms than that used by the negative school. Unfortunately the

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\* I have examined fluid removed from the inflamed joints in rheumatic fever in four cases only, in each instance with a negative result. These observations were considered to be too few for inclusion in this report.

actual technique employed is too seldom specified by authors who are recording their experiences. But from the fact that Beaton and Walker mention that several of their positive results were obtained with blood drawn from the lobe of the ear it may be inferred that no special method has been found necessary by these two observers; and presumably quite a small quantity of blood sufficed for the cultivations. On the other hand, it will be seen by a glance at the positive results here recorded in the blood cultures undertaken in cases of malignant endocarditis that the technique adopted is not at all unfruitful of results in general. By this technique Pfeiffer's bacillus has been repeatedly isolated from the circulation and this fact would seem to speak for the success of the method as a means of discovering in the blood micro-organisms which require some coaxing before they reveal themselves. The explanation of discordant results by difference of technique can therefore on these grounds scarcely apply.

(ii.) The cases selected as instances of rheumatic fever may differ in the two sets of observations. I am disposed to regard this as being the true explanation of the discrepancy. To some physicians the fact that a positive blood-culture was reported in a case thought to be rheumatic fever would unhesitatingly lead them seriously to review the diagnosis if not straightway to change it. This attitude is not, of course, strictly logical. Recent observations have gone to show that the presence of micro-organisms in the blood need not always be regarded as the *malum signum* that it formerly was. They have also shown that micro-organisms exist in the general blood stream in some diseases which were previously considered to be due to a purely local infection. These facts do not alter our clinical conceptions of pneumonia and of typhoid fever respectively, neither would the discovery of micro-organisms in the blood of a patient suffering from rheumatic fever of itself change the clinical picture presented by this disease. But it so happens that no criterion exists by which we can define rheumatic fever. It is true that, as already stated, the same symptom-complex is repeated so frequently and so constantly that we feel confident we are dealing with a "specific" disease owning a "specific" cause. But any or all of the separate symptoms present in the disease may occur as the result of infection by one or other of several micro-organisms of known kind. The isolation of one of these micro-organisms, therefore, if it occur but seldom in connection with the set of symptoms referred to, and if it be a manifestly different micro-organism upon different occasions, justifies the diagnosis being made in terms of such bacteriological finding rather than in terms of a disease the essential cause of which is under discussion.

In actual practice the disease which simulates severe rheumatic fever with endocarditis most closely is malignant endocarditis. It is for this disease that the diagnosis of "acute rheumatism" is usually changed as soon as micro-organisms are discovered in the blood. The discovery introduces a gravity into the prognosis of the case which no other observation connected with the patient

can possibly do. If these cases classed as malignant endocarditis were, as a group, indistinguishable clinically from the majority of cases of rheumatic fever and if the micro-organisms isolated from them were in all instances the same, this change of nomenclature would not be justified. Rather might it be said that the first step would have been taken towards proving rheumatic fever to be the result of a specific microbic infection. But neither of these considerations actually applies. Cases designated malignant endocarditis do present, as a class, well marked differentia from cases of rheumatic fever, if not early in their course, then late, and the clinician is usually justified both of his diagnosis and of his prognosis. And the micro-organisms isolated from the cases are not the same, but include several very different species.

It will readily be seen that if this distinction between rheumatic fever and malignant endocarditis be over-ridden the fact that some observers find micro-organisms to be present in the blood in "rheumatic fever" and others do not is thus explained. The cases yielding positive results, if this explanation be true, have been cases of malignant endocarditis. That several of these cases have been of this nature is quite certain from the fact that they are spoken of as showing "fungating endocarditis" post-mortem. In several of the cases described as giving positive results on blood culture no detailed description is given of the state of the endocardium found at the autopsy. In some of the remaining cases no post-mortem examination was obtained. The introduction of the term "malignant endocarditis" by Poynton and Paine suggests an attempt to bridge over this difficulty. This designation has been given by these observers to certain cases which, though on clinical as well as on morbid anatomical grounds undoubted cases of malignant endocarditis, yielded cultures of the "*diplococcus rheumaticus*" on bacteriological examination.

I have already referred to the fact that there are no definite clinical criteria whereby to define rheumatic fever. Neither are there any definite pathological criteria by which to define the disease in the post-mortem room. It is frequently said that the absence of suppuration in connection with the various lesions present in a case of serous membrane inflammation is characteristic of rheumatic fever. Infarcts in various organs are often considered to be due to "rheumatic" emboli if they present no evidence of suppuration. If a micro-organism produces non-suppurative endocarditis and pericarditis with arthritis when injected into rabbits, this is, again, regarded as valuable evidence that the micro-organism is causal for rheumatic fever. But post-mortem experience shows that suppurating infarcts are the exception rather than the rule in malignant endocarditis as well as in rheumatic fever. When they do occur the micro-organism is usually either *streptococcus pyogenes* or *staphylococcus aureus*. And, as already stated, *S. pyogenes* is rarely present in malignant endocarditis apart from surgical cases of the disease. Absence of suppuration, therefore, only suggests that the infecting micro-organism is neither of these two last named cocci; it does not prove anything with regard to specificity of the actual micro-organism present.

The question of the microbic origin of rheumatic fever must be settled, after all, by bacteriological investigation. Much of the deadlock introduced by the existence of these two schools of observers would be removed if we were given some adequate characterisation of the particular micro-organism isolated by the positive school. That the "*diplococcus rheumaticus*" belongs to the streptococcus group is now admitted by its sponsors. But very few efforts have been made to place it definitively within this group. Apart from the clotting of milk and the ready production of acid by the coccus—two features common to a large number of streptococci—we are told nothing of its bio-chemical reactions. In the course of our investigations into the pathogenic streptococci along lines suggested by Gordon, Dr. Andrewes and I had opportunities of examining two strains of the genuine "*diplococcus rheumaticus*." More recently, Dr. Andrewes has examined a third strain, and I am able to add his results to those already obtained by us. The reactions yielded by these three specimens were as follows:—

TABLE XVI.

—		Milk Clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Selicin.	Conferlin.	Mannite.	Growth on Gelatin at 20° C.	Morphology in Broth.
" <i>Diplococcus rheumaticus</i> ,"												
Strain I. . .	..	+	-	-	+	-	-	+	+	-	Good	Brevia.
Strain II.	..	+	+	+	+	-	-	+	+	+	"	"
Strain III.	..	+	+	+	+	-	-	+	+	+	"	"

On comparing these reactions with the type reactions set out in Table IX. it will be seen that they are those of *streptococcus faecalis*, to which type, therefore, the "*diplococcus rheumaticus*" appears to belong. But these are also reactions yielded by streptococci frequently isolated from the blood in cases of malignant endocarditis (cf. Table XI). This fact of itself goes far to confirm the notion that the cases of "rheumatic fever" which have yielded micro-organisms on blood culture have in reality been cases of malignant endocarditis.

I now pass to the experimental section of this investigation. The production of endocarditis and other serous membrane inflammations by the injection of the "*diplococcus rheumaticus*" is held to be evidence of the causal relationship of this micro-organism to rheumatic fever. But the production of similar lesions by the injection of salivary and faecal streptococci derived from the healthy human alimentary canal, as here detailed, forms an important control experiment. Micro-organisms from sources definitely non-rheumatic were shown by Cole to have the power of causing endocarditis in rabbits. But it is here shown that

similar results may be produced by micro-organisms which are not (to man) pathogenic at all. If Table XVI. and Table XIII. be compared it will be seen that the reactions given by Strain I. of the "diplococcus rheumaticus" are actually identical in all respects with those given by the Colony (A) of faecal streptococcus which caused fatal experimental endocarditis in the rabbit. This was not known at the time the colony was isolated and the injections given. In Table XVII. are set out, for more easy comparison, the reactions given by a streptococcus isolated from a case of malignant endocarditis, those given by a strain of the "diplococcus rheumaticus," and those given by the faecal streptococcus which caused endocarditis and pericarditis in the rabbit. They are all three identical.

TABLE XVII.

	Milk Clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Conferin.	Mannite.	Growth on Gelatin at 20° C.	Morphology in Broth.
I. Streptococcus isolated from a case of malignant endocarditis by blood culture.	+	-	-	+	-	-	+	+	-	Good	Brevis.
II. "Diplococcus rheumaticus."	+	-	-	+	-	-	+	+	-	"	"
III. Streptococcus isolated from normal human faeces and causing fatal endocarditis in rabbits.	+	-	-	+	-	-	+	+	-	"	"

These pathogenic effects set up in rabbits by the injection of streptococci which are saprophytic for man demonstrate the care with which the results of animal experiments should be interpreted. They are also significant as showing that no sharp line can be drawn between "pathogenic" and "saprophytic" micro-organisms. The bearing of these experimental results upon the etiology of rheumatic fever can only be properly assessed by further research. It is possible that rheumatic fever is a form of acute intoxication resulting from increased susceptibility of the host to its own salivary or faecal streptococci. Or it may be that the original *materies morbi*, though of microbic origin, is not a streptococcal poison. Or, again, the virus of rheumatic fever may not be of microbic manufacture at all. The problem is difficult, and its final solution has not yet arrived. These data with regard to the micro-organisms associated with rheumatic fever and malignant endocarditis, and the relations which they bear to those micro-organisms whose sources have been already established, are offered as a contribution to our knowledge on a most important subject.

## APPENDIX B, No. 7.

## PRELIMINARY REPORT on the ACTION of the STREPTOCOCCUS FÆCALIS, and of its CHEMICAL PRODUCTS; by SIDNEY MARTIN, M.D., F.R.S.

*Streptococcus fæcalis* is a micro-organism which is found normally in human fæces, as well as in the fæces of other animals, *e.g.*, the horse.

The researches of Dr. M. H. Gordon\* have shown that the term streptococcus is applied to many kinds of micro-organisms, which, though they may all have the common appearance of a coccus in longer or shorter chains, can be differentiated by their varying effects, when grown in milk, and in solutions containing various sugars and allied bodies (saccharose, lactose, raffinose, inulin, salicin, coniferin, mannite). Milk is coagulated and rendered acid by some, and the sugars and glucosides are fermented and the liquids made acid. This constitutes a "chemical" differentiation of the streptococci, and the application of the method is a valuable means of study of these micro-organisms. This method must, however, be supplemented by their pathological or pathogenic differentiation, which has been the subject of work by F. W. Andrewes and T. J. Horder.† Although classically associated with suppuration, these observers found that in the human being not only suppuration and pyæmia may be the result of streptococcus infection, but cystitis, erysipelas and cellulitis, non-suppurative peritonitis, septicæmia, malignant endocarditis and sore throats may be caused by one or other form of streptococcus, and their work has demonstrated the great value of determining the form of streptococcus which is the cause of the particular infection.

The streptococcus pyogenes, the most virulent of the group, is from its pathogenicity and its vegetative characters in marked contrast with streptococcus fæcalis, which is of low pathogenicity, and possesses active vegetative growth outside the body. The typical streptococcus pyogenes does not coagulate milk nor does it have any effect on neutral red, nor on raffinose, inulin, coniferin, and mannite; while it ferments saccharose, lactose, and salicin. It grows with difficulty in artificial media and soon dies in subculture. The streptococcus fæcalis, on the other hand, coagulates milk and ferments saccharose, lactose, salicin, coniferin, and mannite; while raffinose and inulin are sometimes affected. It grows well in artificial media and does not readily die in subculture.

The streptococcus pyogenes is a highly virulent micro-organism to man and rodents, although outside the body it rapidly loses its pathogenic power; the streptococcus fæcalis is not pathogenic to

\* Report of Medical Officer, Local Government Board, Vol. XXXIII, p. 388.

† "Lancet," September, 1906.

mice (Andrewes and Horder). It has been found in the human being in some cases of cystitis, otitis media, septicæmia, and malignant endocarditis, and its study is, therefore, of importance to the occurrence of disease in the human body.

The work recorded in this report was done with two forms of streptococcus *fæcalis*, kindly sent to me by Dr. F. W. Andrewes, of St. Bartholomew's Hospital. The first of these was from a stock which has been called a "rheumococcus" (Poynton and Paine). It gave the typical chemical reactions of the streptococcus *fæcalis*, viz., coagulating milk and fermenting mannite, lactose, salicin, and saccharose; while inulin and raffinose were not effected. These reactions were repeated by me, with the same results, except that I thought that there was some reaction in its growth in the inulin solution. The second stock of streptococcus *fæcalis* as tested by me gave reactions nearly identical with the first, with the exception that raffinose was fermented by it.

Microscopically both forms showed cocci in chains of 2, 4, 6, and 8, sometimes singly. The staining reactions were those of cocci generally, viz., by all the ordinary anilin stains and by Gram's method.

*Vegetative properties of the streptococcus fæcalis.*—The coccus grows well in the ordinary media, broth, and agar, and may be subcultured for months without losing any of its cultural or chemical characteristics. It differs in this way markedly from the streptococcus *pyogenes*.

#### *Pathogenic effects in Animals.*

1. *In mice.*—To mice the streptococcus *pyogenes* is highly pathogenic. The effect of streptococcus *fæcalis* when tested on these animals was found to be negative as regards the production of an infective process.

*Experiment 1.*—A mouse was injected subcutaneously with 0.5 cc. of a broth culture 7-8 days. The animal became ill in 24 hours, and died in 6 days. P.m.—No lesions could be found, and cultures made from the heart's blood were negative.

*Experiment 2.*—A small quantity of the growth on agar was injected subcutaneously in a mouse. No illness was produced, and the animal remained alive and well for 24 days, when it died. Cultures made from the spleen and heart's blood were negative.

*Experiment 3.*—Two mice were injected subcutaneously with a small quantity of broth culture. One survived; the other died in 13 days, but no streptococcus was obtained from the spleen or blood.

*Experiment 4.*—Three mice were injected subcutaneously with small quantities of an agar culture. They were all alive and well 7 weeks afterwards.

In mice, therefore, there is no evidence that the streptococcus *fæcalis* produces an infective process. Although some of the animals died, the cause of death was in all probability not an infection by the micro-organism, as this was not obtained by culture after death.

2. *In rabbits.*—In rabbits the streptococcus *fæcalis* is pathogenic, causing death by producing (1) a septicæmic condition

and (2) the primary lesions of malignant endocarditis. This is shown in the following experiments.

The cultures injected were made on agar in the following way :—A 24-hour-old broth culture was inoculated by means of a pipette on the surface of a slope-agar tube and the liquid allowed to run over the surface of the agar, so that a uniform growth occurred over the whole surface. After 3–4 days' incubation at 37° an emulsion was made of the culture with sterile normal NaCl solution.

*Experiment I.*—A rabbit, weighing 2,130 grammes, received intravenously an emulsion of the growth from 6 agar tubes. The animal became ill in under 24 hours, the temperature falling from 102° F. to 98° in about 15 hours, still further to 96·6° in 42 hours, death occurring in about 80 hours, the temperature being below 95° for several hours before death. This result, with the effect on the body temperature, is more comparable to the effect of a chemical bacterial poison than to that of an infection; and after death the cultures from the heart's blood were negative. The animal was well nourished and showed some hæmorrhage into the cortex of the left suprarenal body, as well as some adhesions between the pericardium and the anterior chest wall. The pericardium contained about one drachm of slightly turbid yellowish fluid; but there was no endocarditis.

*Experiment II.*—A rabbit weighing 2,320 grammes received intravenously an emulsion of the growth from 6 agar tubes. The effects on the body temperature and weight are shown in the following table :—

*Rabbit.*—2,320 grammes. Intravenous injection of *Streptococcus faecalis*.

—	—	Rectal Tempera- ture.	Weight in Gramme.	—
June 24, 1907	P.M.			
	3. 0	102·2	2,320	
	3.30	Injection made into marginal vein of left ear.		
	5.15	104	—	} Period of pyrexia, 7 days.
" 25, "	9.30	105·2	—	
	12. 0	105·6	—	
	6. 0	105·4	—	
" 26, "	9. 0	104·4	—	
	1.30	104·8	—	
	5.30	105·4	—	
" 27, "	9. 0	104·4	2,130	
	1. 0	104·6	—	
	5.30	104·4	—	
" 28, "	9. 0	104·4	—	
	1. 0	104·2	—	
	6. 0	104·2	—	
" 29, "	9. 0	104·2	—	
	12.30	104·4	—	
July 1, "	9. 0	104	1,880	
	2. 0	103·6	—	
	6. 0	103·8	—	

—	—	Rectal Tempera- ture.	Weight in Grammes.	—
July 2, 1907	P.M.			
	9. 0	103	—	
	2. 0	102.8	—	
" 3, "	6. 0	102.4	—	
	9. 0	102.2	—	
	12. 0	102.4	—	
" 4, "	5.30	102	—	
	9. 0	101.8	1,770	
	12. 0	101.8	—	
" 5, "	5.45	101.6	—	
	9. 0	101	—	
	1.30	101.4	—	
" 6, "	5.30	101.2	—	
	9. 0	100.6	1,660	
	12.45	100.2	—	
" 8, "	9. 0	100	—	
	1.30	100	—	
	5.30	100.4	—	
" 9, "	9. 0	99.4	—	
	12. 0	99.4	—	
	5. 0	99.6	—	
" 10, "	9. 0	99	1,565	
	1.30	99.2	—	
	5.30	99.4	—	
" 11, "	9. 0	99	—	
	1. 0	98.4	—	
	6. 0	95.6	—	
" 12, "	9. 0	Found dead	1,430	

Period of gradual fall of temperature to sub-normal, 11 days.

Duration of life after inoculation, 18 days.

Total loss of weight, which was gradual, 890 grammes.

*Post mortem examination.*—The body was thin and emaciated :

*Pericardium and pleuræ* contained a moderate quantity of clear effusion.

*Heart* showed whitish nodule vegetations on the mitral valves, at the edge of the valve and on the cordac tendinæ. There were a few small nodules also on the tricuspid valve, but the pulmonary and aortic valves were normal. On the endocardium of the septum on each side was an area of roughening due to vegetations, a similar lesion to that frequently observed in malignant endocarditis in the human being. The heart muscle was pale, but otherwise normal.

*Lungs.*—No naked eye change.

*Spleen.*—Not enlarged ; pale.

*Kidneys and liver* pale. No effusion in the peritoneal cavity. The surface of the liver was rough from a slight and recent deposit of lymph.

*Cultures* were made from the blood in the right auricle and right ventricle in broth and on agar. They all remained sterile. A small nodule on the mitral valve

was cut off and placed in broth. After incubation the broth became turbid, and a subculture on agar gave a pure growth of the streptococcus fæcalis.

*Results.*—The inoculation of the streptococcus fæcalis in this rabbit produced a septicæmic condition, with death in 18 days. An endocarditis of both tricuspid and mitral valves was produced by the streptococcus, which was recovered from the lesions. The results were obtained from one injection only. No embolism or infarction was found as the result of the endocarditis, possibly because the condition had not lasted sufficiently long. This question, as well as the further pathogenic effects of the streptococcus, is now being investigated.

#### *Chemical Product of the Streptococcus.*

The investigation of the chemical products and poisons of the streptococcus was begun with two points in view: that the poisons may be present (1) in the bodies of the cocci, as well as in (2) the culture fluid of one or other composition in which the cocci were grown.

1. *Examination of the toxic agents in the bodies of the cocci.*—The cocci were obtained in mass in the following way:—six flasks, each containing 100 cc. of broth, were inoculated from an agar culture of the streptococcus and incubated for 17 days. Each flask was then tested to see if the growth was pure. The liquid was then centrifugalized, the deposit of cocci washed to get rid of any culture liquid and dried *in vacuo* over  $H_2SO_4$ . In another experiment 12 large agar plates were inoculated with a broth culture, the liquid being allowed to flow over the agar so as to get a large surface growth. The purity of the growth, which was allowed to continue for four or five days, could readily be seen under a lens. The surface growth was washed off with sterilized distilled water and the liquid centrifugalized; the deposit was dried *in vacuo* over  $H_2SO_4$ .

In this way 0.09 gramme of dried cocci was obtained. The scaly dried product was ground up in a mortar, made into an emulsion with sterile normal NaCl solution and used for injection into a rabbit.

The rabbit weighed 1,800 grammes. The injection was made into the marginal vein of the ear, and symptoms were soon produced. Before the injection the temperature was  $102.2^\circ$ ; in one hour and a quarter afterwards it was  $96^\circ$ ; in seven hours it was below  $95^\circ$ , and the animal was unconscious; it died soon afterwards. There were no convulsions, and no other symptoms than those mentioned.

*Post mortem.*—The animal was well nourished. There was a small amount of fluid in the pleuræ and peritoneum. The lungs were somewhat oedematous. Nothing further abnormal was observed in the organs, except some pallor of the liver, spleen, and kidneys.

There is no doubt, therefore, that in the bodies of the streptococcus there is a powerful poison (endotoxin), the properties of which will require further investigation.

2. *Growth of the streptococcus in liquid media.*—The investigation of any chemical products which may be excreted by the streptococcus or may be formed by it in suitable media has only just been begun. Some months were spent in testing various media, containing a digestible proteid (alkali-albumin), as it was found that the streptococcus was sensitive in its growth as regards not only the amount of proteid present but also, and to a greater extent, to the degree of alkalinity of the medium.

Thus liquid serum in which the proteids are transformed into alkali albumin by the addition of caustic soda which is subsequently neutralized was found to be an unsuitable media. The streptococcus grew in it, but only feebly, and tended to die out.

After many trials the following medium was devised, and in it the coccus grew well for nearly six weeks (when the experiment ended), and was as vigorous in its vegetative growth at the end of the experiment as at the commencement.

The medium was prepared by adding caustic soda to liquid (filtered) serum, keeping at 37° C. for 24–48 hours, then boiling. By this means the proteids are transformed into alkali albumin. This is now precipitated from solution by adding HCl; the precipitate is collected and dissolved in 2 per 1,000 NaHO and boiled.

This solution of alkali albumin is mixed with acid broth (made from meat) and neutralized to the alkalinity of the ordinary broth culture medium.

With this culture liquid experiments are now in progress; but no results have been obtained which can as yet be brought forward.

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## APPENDIX C.

PRELIMINARY REPORT on the RESULTS OF SUSTAINED SUBJECTION of GLYCERINATED CALF LYMPH to TEMPERATURES below FREEZING POINT; by DR. FRANK R. BLAXALL and MR. H. S. FREMLIN.

These experiments were in the first instance undertaken to determine whether the active agent of vaccine lymph could withstand, without loss of potency, temperatures below the freezing point. Afterward, they were directed to ascertain whether sustained subjection of glycerinated calf lymph to a temperature 5° (Centigrade) below freezing point could be utilised as a means of maintaining a reserve and store of lymph without risk of loss of its specific activity.

General experience and numerous special experiments have shown that the agents most productive of weakness and failure in glycerinated lymphs are *age* and *heat*. Thus, the active "vaccine" agent present in crude lymph when collected from the calf loses its potency if subjected for five minutes\* to a higher temperature than 57·5°C. Again, glycerinated lymph becomes vaccinally inert if kept at 37°C. for more than 36 hours. Similarly, the extraneous micro-organisms apt to be present in glycerinated lymph are eliminated the more quickly the higher the temperature to which the lymph is exposed.† At 37°C., for instance, their elimination is accomplished in 36 hours or less; contrariwise, at 10°C., and at lower temperatures, their elimination is very slow. It was anticipated, therefore, that if the everyday deleterious influence of heat on lymph could be neutralised, such tendency to loss of activity—which in present conditions is associated with "age" of lymph—might be greatly mitigated, and a method of storing glycerinated lymph with full preservation of its potency thus obtained.

Our first experiments were commenced in January, 1900, with "freezing mixtures." Samples of glycerinated lymph contained in small test tubes hermetically sealed were placed in a mixture of ice and salt. The temperature to which the tubes were thus subjected was found to range in practice from 9 degrees below to 10 degrees above zero, Centigrade; and no means being at hand of obtaining a low, and at the same time stable, temperature, the samples were at the end of a week withdrawn from the "mixture." These samples were then tested on calves, side by side with other samples of the same glycerinated lymph which throughout the experiment had been kept in an ice chest at 10°C. Excellent vesicles were

\* Medical Officer's Report to the Local Government Board, 1900-01, p. 634.

† Medical Officer's Report to the Local Government Board, 1902-03, p. 649.

developed by both sets of samples, showing that the vaccine organism had not suffered any impairment of potency by an exposure of uncertain duration to a temperature of  $-9^{\circ}\text{C}$ .

Our second experiment was to subject samples of glycerinated lymph to a much lower temperature, namely, that of liquid air, which is the equivalent of  $-180^{\circ}\text{C}$ . This we were enabled to do by the courtesy of Dr. Macfadyen and Mr. Rowland of the Lister Institute.

Samples from four glycerinated lymphs were placed in leaden capsules each holding about 3 c.c., and these were inserted in a vessel containing liquid air; companion samples of the same glycerinated lymphs being kept as controls in an ice chest at  $10^{\circ}\text{C}$ . At intervals of 1, 2, 3, 5, and 11 weeks the samples were withdrawn from the liquid air for the short time necessary for allowing capillary tubes to be charged from them, similar procedure being adopted with the corresponding samples in the ice chest. These capillary tube samples of the lymphs were forthwith tested on calves to ascertain whether their potency had been affected. At the end of 11 weeks, exposure of the samples to liquid air ceased, but the experiment was prolonged by further storage, alike of experimental and control samples, in an ice chest at  $10^{\circ}\text{C}$ . for an additional 15 weeks, with a view to testing from time to time, and at the end of that period, samples of both sorts on calves.

The results of the inoculations of the samples on calves were as follows:—

Up to 11 weeks, the limit of the period of subjection of samples to the temperature of liquid air, both sets of samples gave equally good vesicles on calves; no difference was distinguishable between them. Thus, sustained exposure to the temperature of liquid air for near upon three months had in no way impaired the potency of the glycerinated lymphs. On the other hand, the results obtained with the two sets of samples after the additional storage for 15 weeks at  $10^{\circ}\text{C}$ . were very different. Those samples which had in the first instance been exposed to the temperature of liquid air continued, after 15 weeks additional storage at  $10^{\circ}\text{C}$ ., to yield good vesicles on the calf, whereas the control lymphs yielded, after further keeping in like fashion, only poor vesicles, or were quite inert. *It appeared indeed that advantage had accrued to those sections of the glycerinated lymphs which had been subjected for 11 weeks to the temperature of liquid air.*

Our third experiment was on a larger scale. It consisted in placing glycerinated lymph for a year in a "Cold Storage Dépôt" in London, in which the temperature was held to be maintained with great regularity at  $22^{\circ}\text{Fahr.}$  or  $-5^{\circ}\text{C}$ . Samples from 92 glycerinated lymphs were taken for test in this sense. These samples at the date of the experiment varied greatly in age; the oldest was 8 months and the youngest 16 days, from the date of collection. Two samples, each consisting of about 1 c.c. of emulsion, were taken of each

lymph; and these were placed in separate small test tubes, corked and sealed. Each such test tube was then placed in a wooden case, and this case was tightly corked. One set of 92 samples was placed in a sealed package and inserted in the Cold Store Dépôt on October 3rd, 1902, and the other (companion) set, on the same date, was placed in an ice chest at 10°C. in our Laboratories. There they remained in each instance for one year. On October 2nd, 1903, these packages were withdrawn from cold store and from ice chest, and their contents were examined. All the samples which had been stored at -5°C. were in good condition, but in the case of many of those which had been stored at 10°C., the emulsion had to a considerable extent soaked away or dried up. The several samples were now tested for potency on calves, inoculations being made on three separate occasions on these animals within a period of three weeks, the samples being meanwhile stored at room temperature. On each occasion each "cold store" sample was tested side by side with its companion "ice chest" sample, on one and the same calf. As a result, the "cold store" lymphs yielded 91.4 per cent. "insertion success," whereas the "ice chest" lymphs yielded no more than 16.2 per cent. (See Table I. annexed.) It deserves to be noted, moreover, that the failures with the "cold store" lymphs occurred only when these having been three weeks removed from the store had been further kept at room temperature. It was observed, too, that the vesicles yielded on the calves by the "cold store" lymphs were of better appearance and quality than those yielded by the "ice chest" lymphs.

A fourth experiment on the same lines was made with 91 glycerinated lymphs. Two samples of each, taken as before, were placed in small glass test tubes, which were then corked and sealed, and each such test tube was in turn packed in a wooden case duly corked. On this occasion each set of samples was, in addition, enclosed in a tin box, the lid of which was soldered on, so that the box was hermetically sealed; the purpose being to obviate, if possible, the drying up of emulsions such as had occurred in the third experiment in the case of samples stored in the ice chest. Of these 91 glycerinated lymphs, the oldest at the date of the experiment was 13 months from the time of collection, and the youngest nine days.

The tin boxes remained respectively in the cold store at -5°C. and in an ice chest at 10°C. for a whole year, at the expiration of which period the boxes were withdrawn and the contents examined. As before, the samples which had been in the cold store were found in good condition, and on this occasion drying up of those which had remained in the ice chest at 10°C. had to a considerable extent been checked, if not entirely prevented. All the samples were inoculated on calves, in the same way as before, on three occasions, the last inoculation being made 32 days after removal of the tin boxes from the cold store and from the ice chest respectively;—the samples having been kept, after removal from the tin boxes,

meanwhile in an ice chest at 10°C. As a result of inoculation of calves the "cold store" lymph yielded 98.9 per cent., and the "ice chest" samples 41.8 per cent., insertion success. And, as before, it was noted that the vesicles yielded by the "cold store" samples were of better appearance and quality than those yielded by samples from the "ice chest." (See Table II. annexed.)

The above two series of experiments showed definitely enough that, as tested on calves, cold storage of glycerinated lymph for a year at -5°C. prevents that deterioration and loss of potency which commonly occurs to lymph stored at the higher temperature 10°C. Bacteriological examinations were made of all these samples at the time of withdrawal from the cold store at -5°C., and ice chest at 10°C., respectively. The results showed that storage in glycerine for a year or more, whether at -5°C. or 10°C., serves to practically eliminate the contained extraneous organisms.

At this stage, therefore, it was sought to ascertain the effect of the cold storage treatment on lymph subsequently employed in human vaccination. For this purpose 14 glycerinated lymphs contained in small sealed test tubes were packed in a hermetically closed box, placed in the cold store at -5°C. and were retained there for 4 months and 23 days. When withdrawn, these lymphs were examined and tested for potency in the usual way. These preliminary tests having proved satisfactory, the 14 lymphs were issued to Public Vaccinators, on an average 19 days after removal from the cold store. Employed by Public Vaccinators in the vaccination of 16,771 individuals they gave a percentage success of—

Case	...	...	...	98.8
Insertion	...	...	...	94.2

slightly higher figures than the average success rate obtained in the preceding year with the current lymph of the Establishment, viz., 98.4 per cent. case, 93.5 per cent. insertion, success. The current lymph of the previous year had been at the time of issue "aged" one month to two months, so that the cold store lymph in spite of its greater age gave higher percentage results than the lymph ordinarily issued.

From these experiments it would appear:—

(1) That, in glycerinated lymph, the active agent of vaccine not only can withstand freezing but can survive a temperature 180°C. below freezing point for a considerable time, and this without loss of potency.

(2) That glycerinated lymph can be retained in cold store at -5°C. for a year, without diminution of its potency, whereas glycerinated lymph, stored at 10°C. for a year, parts with its activity to an uncertain but considerable extent.

(3) That sustained subjection to cold appears to be in no sense hostile to the active agent of vaccine; that, on the contrary, lymph thus dealt with was capable of producing excellent vesicles on calves, and that the results obtained with it in human vaccination were wholly satisfactory.

TABLE I.

Results on calves of glycerinated lymph subjected to a temperature of  $-5^{\circ}\text{C}.$  in a cold store, or to  $10^{\circ}\text{C}.$  for 12 months, 1902-1903 :—

Calves Inoculated.	Date of Experiment.	Results on Calves.			
		Cold Store, $-5^{\circ}\text{C}.$		Ice Chest, $10^{\circ}\text{C}.$ (Control)	
		Insertions :—		Insertions :—	
		Taken.	Failed.	Taken.	Failed.
1 ... ..	2nd October, 1903 ... ..	45	0	0	45
2 ... ..	9th October, 1903 ... ..	39	0	13	26
3 ... ..	23rd October, 1903 ... ..	12	9	4	17
	Total ... ..	96	9	17	88
	Percentage success ...	91·4		16·2	

TABLE II.

Results on calves of glycerinated lymph subjected to a temperature of  $-5^{\circ}\text{C}.$  in a cold store, or to  $10^{\circ}\text{C}.$  for 12 months, 1904-1905 :—

Calves Inoculated.	Date of Experiment.	Results on Calves.			
		Cold Store, $-5^{\circ}\text{C}.$		Ice Chest, $10^{\circ}\text{C}.$ (Control).	
		Insertions :—		Insertions :—	
		Taken.	Failed.	Taken.	Failed.
1 ... ..	17th March, 1905 ... ..	90	1	40	51
2 ... ..	31st March, 1905 ... ..	89	2	37	54
3 ... ..	14th April, 1905 ... ..	91	0	37	54
	Total ... ..	270	3	114	159
	Percentage success ...	98·9		41·8	





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